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TITLE: Synthetic Beta-Lactam Antibiotics as a Selective Breast Cancer Cell Apoptosis Inducer: Significance in Breast Cancer Prevention and Treatment

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14. ABSTRACT Activation of the cellular apoptotic program is a current strategy for the prevention and treatment of human cancer including breast cancer. Because of the ease of synthesis and structural manipulation, small molecules with apoptosis-inducing ability have great potential to be developed into chemotherapeutic drugs. The b-lactam antibiotics have for the past 60 years played an essential role in treating bacterial infections without causing toxic side effects in the host. We hypothesized that active N-thiolated b-lactams can target a tumor-specific protein(s) and selectively induce apoptosis in human breast cancer but not normal cells. In this report, we have designed and synthesized a number of beta-lactams with selected C3 and N1 ring substituents, and evaluated their potencies to inhibit proliferation and induce apoptosis in human breast cancer cells. We have also studied the biochemical targets of these b-lactams by performing microarray assay. Our results supported by this IDEA award strongly support our hypothesis that beta-lactams cause tumor DNA damage, which is responsible for their anti-tumor activities. Our studies have provided strong support for proof-of-concept of the potential use of these b-lactams in breast cancer prevention and treatment.					
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A STATEMENT FROM THE PRINCIPAL INVESTIGATOR

The Annual (Fifth, Revised) summary report of this grant was just approved on December 21, 2007 (see included document). On February 1, 2008, I was notified that an additional \$3,443.38 has been returned by my former employer (H. Lee Moffitt Cancer Center) to the Government for this award (see included documents).

Based on the above information, I would like to remind the reviewers that **the fund for supporting the research for the period 1 MAR 2007 - 28 FEB 2008 is not in the same as that of previous years.**

Therefore, in this report, I will just report some new results generated most recently. Thanks!

INTRODUCTION

Activation of the cellular apoptotic program is a current strategy for the prevention and treatment of human cancer including breast cancer (1). Because of the ease of synthesis and structural manipulation, small molecules with apoptosis-inducing ability have great potential to be developed into chemotherapeutic drugs (2). One particularly important class of small molecule drugs, the beta-lactam antibiotics, have for the past 60 years played an essential role in treating bacterial infections without causing toxic side effects in the host (3, 4). Several years ago we uncovered new members of this family of drugs, termed N-thiolated beta-lactams, which are highly effective at inhibiting bacterial growth in drug-resistant strains of *Staphylococcus aureus* (5, 6). Their mode of action appears to differ from that of traditional beta-lactam antibiotics. Most innovatively, we have discovered and characterized, for the first time, the anti-proliferative and apoptosis-inducing properties of N-thiolated beta-lactam antibiotics against human tumor cells (7, 8). Based on these results, we hypothesized that active N-thiolated β -lactams can target a tumor-specific protein(s) and selectively induce apoptosis in human breast cancer but not normal cells. To test this innovative hypothesis, we have performed the proposed experiments as reported below.

BODY

For details, please see the included **APPENDICES**. Please also see below **KEY RESEARCH ACCOMPLISHMENTS**.

Induction of tumor cell apoptosis by a novel class of N-thiolated β -lactam antibiotics with structural modifications at N_1 and C_3 of the lactam ring (9 and see Appendices). The investigation of novel anti-tumor agents that preferentially select for malignant cells with a tolerable toxicity level has been the focus of anti-cancer drug discovery. Our laboratories have previously reported that certain N-alkylthiolated β -lactams had DNA-damaging and apoptosis-inducing activity in various tumor lines but not in nontransformed cells. In the current study we further delineated the effects of substitutions at C_3 or N_1 of the lactam ring for cell death-inducing capability with close attention paid to a discernible structure-activity relationship (SAR). We found that two β -lactam analogs (JG-5 and JG-19), both containing a

branched-chain moiety at C₃ of the lactam ring, exhibit potent apoptosis-inducing activity. Additionally, JG-5 exhibited superior in vitro biological activity over JG-19 owing to structural modifications made to substituents at the N₁ and C₃ positions of the lactam ring. Our results strongly warrant further investigation into these novel β -lactams as potential anti-cancer therapeutics.

GADD45 β and HSP-70 are two of the main targets for L-1 in Jurkat T cells (10 and see Appendices). We have previously reported that *N*-thiolated β -lactams have the ability to induce DNA damage and apoptosis selectively in transformed and malignant, but not in non-transformed cells. However, the mechanisms for DNA-damaging and apoptosis-inducing effects, as well as for the selectivity of these β -lactams, are still unknown. In an attempt to answer these questions, we used microarray assay to analyze the gene expression profile in Jurkat T cells following Lactam-1 (L-1) treatment. To select time points for microarray analysis, we were specifically interested in the cells that have already sustained DNA damage but have not undergone apoptosis, in comparison to the cells in which the apoptosis has already started. A time course experiment in which Jurkat T cells were treated with 60 μ mol/L of L-1 was conducted and the cells were analyzed for cell viability (Trypan blue dye exclusion assay), DNA damage (TUNEL assay), and PARP cleavage (Western blot). We found that 2 hour treatment of Jurkat T cells with 60 μ mol/L of L-1 resulted in 22% of nonviable cells (see Fig. 1B in the included manuscript reference #10) and induced strong DNA damage (76.3% TUNEL-positive cells) (Fig. 1C), but not apoptosis as confirmed by the absence of cleaved PARP protein (Fig. 1D). However, after 12 hour of treatment, 56% Trypan blue-positive cells (Fig. 1B) and 96.8% TUNEL-positive cells (Fig. 1C) were detected, together with the apoptosis induction confirmed by PARP cleavage (Fig. 1D).

Therefore, we isolated RNA from Jurkat T cells treated for 2 and 12 hours, converted it to cDNA and analyzed by the Affymetrix U133A chips and Affymetrix Microarray 5.0 (MAS 5.0) software. By analyzing over 22,000 genes, we found that expression of many genes was increased by more than 2-fold, following just 2 hours of treatment with L-1. A sample of 13 genes was selected for this study (Table 1). Among them, a number of genes encoding DNA-interacting proteins was significantly affected, including growth arrest and DNA damage inducible gene-45 (GADD45), inhibitor of DNA binding 2 (ID2) and zinc finger 38 (ZF38). The fact that induction of these genes preceded apoptosis suggests that up-regulation of DNA-interacting proteins is an important component of the cellular response to L-1 treatment. An almost 44-fold increase in GADD45 β mRNA expression was detected after 2 hour treatment (Table 1), which was associated with a significant increase in the level of GADD45 β protein after 12 hours of treatment with 60 μ mol/L of β -lactam 1 (Fig. 1E).

The largest transcriptional increase was observed in multiple isoforms of Hsp70 mRNA (e.g., isoform 1A, 67-fold increase after a 2 hour treatment, Table 1). This significant up-regulation of Hsp70 gene transcription was followed by a consequent increase in Hsp70 protein level, starting at 6 hours of treatment, as showed by Western blot assay (Fig. 1D).

L-1 inhibits proliferation and induces apoptosis in human breast cancer MDA-MB-231 cells (10 and see Appendices). Our results demonstrated that L-1 has the ability to induce cell death in leukemia Jurkat T cells (Fig. 1). We next investigated whether the same effect could be observed in solid tumor cell lines, such as human breast cancer MDA-MB-231 cells. We first tested the anti-proliferative potency of L-1, by plating the MDA-MB-231 cells in 96-well plate and treating them with various concentrations of L-1 for 24 hours, followed by an MTT assay. We found that L-1 inhibits proliferation of MDA-MB-231 cells in a dose-dependent

manner (Fig. 2A); at 25 $\mu\text{mol/L}$ concentration L-1 inhibited cell proliferation by approximately 41% (p value < 0.01) whereas at 75 $\mu\text{mol/L}$ concentration it induced 80% inhibition (p value < 0.01). Inhibition of MDA-MB-231 cell proliferation by L-1 was associated with a dose-dependent PARP cleavage (Fig. 2B), which is a hallmark of apoptosis. These data show that the ability of L-1 to inhibit cell proliferation is associated with apoptosis induction not only in leukemia Jurkat T-cells but also in breast cancer MDA-MB-231. After L-1 treatment, the protein levels of GADD45 β and Hsp70 in MDA-MB-231 cells were also increased in a dose-dependent manner (Fig. 2B).

I would like to emphasize that the in vivo experiments reported in references #9 and #10 have been approved by the ACURO office at the Office of Research Protections at the USAMRMC and these animal experiments were supported by the Concept award (W81XWH-04-1-0688).

Development of these beta-lactams into potential antitumor drugs is our long-term goal. These studies should provide strong support for proof-of-concept of the potential use of these N-thiolated beta-lactams in breast cancer prevention and treatment.

KEY RESEARCH ACCOMPLISHMENTS

- Published 2 articles and submitted 1 manuscript
- Gave at least 8 scientific presentations
- Trained three Ph.D. students
- Received a DOD Breast Cancer Research Program-Concept Award (**W81XWH-04-1-0688**) (PI: Q. Ping Dou).
- Received a Training Grant (**T32-CA09531-19 NIH**) (Ms. Kristin R. Landis-Piwowar; Mentor: Q. Ping Dou)
- Received a Training Grant (**T32-CA09531-19 NIH**) (Mr. Michael Frezza; Mentor: Q. Ping Dou)
- NIH R01. N-Thiolated beta-Lactams. Co-Principal Investigator: Q. Ping Dou (PI: Ed Turos).
- Received a NIH R01 Award (**5 R01 CA120009**) (PI: Q. Ping Dou).
- Received a NIH R21 Award (Co-PI: Q. Ping Dou; PI: Jayanth Panyam).
- Partially supported several personnel (Di Chen, Ph.D., Huanjie Yang, Ph.D., Cindy Cui, B.S., Vesna Minic, M.S.)

REPORTABLE OUTCOMES

Provide a list of reportable outcomes that have resulted from this research to include:

Manuscripts (see Appendices):

Kevin D. Revell, Bart Heldreth, Timothy E. Long, Seyoung Jang and Edward Turos. N-thiolated beta-lactams: Studies on the mode of action and identification of a primary cellular target in *Staphylococcus aureus*. *Bioorg Med Chem*. 2007 Mar 15;15(6):2453-67.

Frezza M, Garay J, Chen D, Cui QZC, Turos E and Dou QP. Induction of tumor cell apoptosis by a novel class of N-thiolated β -lactam antibiotics with structural modifications at N₁ and C₃ of the lactam ring. Intl. J Mol Med., 2008; in press.

Di Chen, Samuel C. Falsetti, Michael Frezza, Vesna Milacic, Aslamuzzaman Kazi, Qiuzhi Cindy Cui, Timothy E. Long, Edward Turos, and Q. Ping Dou. Anti-tumor activity of N-thiolated β -lactam antibiotics. Cancer Letter, in revision.

Abstracts/Scientific Presentations:

Dou QP. Invited Speaker. Molecular Prevention of Human Cancer: A Lesson Learned from Diet-Gene-Environment Interaction. Environmental Toxicology Graduate Program and Department of Chemistry, University of California at Riverside, Riverside, CA, April 18, 2007.

Dou QP. Invited Speaker. Molecular Prevention of Human Cancer: Role of Diet-Gene-Environment Interactions. College of Chemistry, Central China Normal University, Wuhan, China, June 29, 2007

Dou QP. Invited Speaker. Molecular Prevention of Human Cancer: Role of Diet-Gene-Environment Interactions. School of Medicine, Shandong University, Jinan, Shandong, China, July 4, 2007

Dou QP. Invited Speaker. Molecular Prevention of Human Cancer: Role of Diet-Gene-Environment Interactions. Department of Pathophysiology, Guangzhou Medical College, Guangzhou, China, July 19, 2007

Dou QP. Invited Speaker. New use of old copper-binding drugs: converting the proangiogenic copper to a specific cancer cell death inducer. Molecular Therapeutics of Cancer, Gordon Research Conference, Colby-Sawyer College, New London, New Hampshire, July 22-27, 2007.

Vesna Milacic (Mentor: Dou QP). Potential use of gold(III) dithiocarbamates as proteasome inhibitors and apoptosis inducers for breast cancer treatment. Wayne State University Graduate Student Research Day, Detroit, MI, September 20, 2007.

Frezza M, (Mentor: Dou QP). Gallium(III) complexes as proteasome inhibitors and apoptosis inducers in prostate cancer cells. Wayne State University Graduate Student Research Day, Detroit, MI, September 20, 2007.

Dou QP. Invited Speaker. Converting the proangiogenic copper to a specific cancer cell death inducer: importance of tumor tissue copper mapping. The joint XOR/BioCAT Microprobe Workshop, Northwestern University, Chicago, IL, November 15-16, 2007

Patents and licenses applied for and/or issued:

None

Degrees obtained that are supported by this award:

Kristin Landis-Piwowar, Cancer Biology Program, Wayne State University, **Graduated with Ph.D. on August, 2007**. Dissertation Title: "Synthetic Green Tea Catechin Analogs and Their Prodrugs: A Structural and Biological Analysis of (-)-Epigallocatechin-3-Gallate Variations" (Advisor: Q. Ping Dou).

Vesna Milacic, Cancer Biology Program, Wayne State University, **Scheduled to defend her Ph.D. degree on August, 2008.** (Advisor: Q. Ping Dou).

Development of cell lines, tissue or serum repositories; infomatics such as databases and animal models, etc:

None.

Funding applied for based on work supported by this award:

DOD Breast Cancer Research Program-Concept Award (**W81XWH-04-1-0688**). Examination of potential anti-tumor activity of N-thiolated beta-lactam antibiotics in nude mice bearing human breast tumors. 5% Effort (PI: Q. Ping Dou). 10/01/04-09/30/06 (no cost extension).

T32-CA09531-19 NIH Training Grant. "Training Program in the Biology of Cancer" (Ms. Kristin R. Landis-Piwowar; Mentor: Q. Ping Dou) 09/01/05-8/31/07.

T32-CA09531 NIH Training Grant. "Training Program in the Biology of Cancer" (Mr. Michael Frezza; Mentor: Q. Ping Dou) 09/01/07-8/31/09.

NIH R01. N-Thiolated beta-Lactams. Co-Principal Investigator: Q. Ping Dou (5%) (PI: Ed Turos). 03/01/02-02/28/07.

NIH R01. Roles of polymorphic COMT, tea polyphenols and proteasome in cancer prevention. 20% Effort (PI: Q. Ping Dou). 04/01/06-03/31/11.

NIH R21. Dual-agent nanoparticles to overcome drug resistance. 5% Effort (Co-PI: Q. Ping Dou; PI: Jayanth Panyam). 07/01/07-06/30/09.

Employment or research opportunities applied for and/or received based on experience/training supported by this award:

Huanjie Yang, Ph.D.

Di Chen, Ph.D.

Cindy (Qiuzhi) Cui, Technician

Kristin Landis-Piwowar (Ph.D. defense on August, 2007)

Vesna Minic, M.S. (Ph.D. student)

CONCLUSIONS

We have designed and synthesized a number of beta-lactams with selected C₃ and C₄ ring substituents, and evaluated potencies of these synthetic beta-lactams to inhibit proliferation and induce apoptosis in human breast cancer cells. To study the biochemical target of these N-thiolated beta-lactams, we performed a microarray assay and found a number of potential target genes regulated by beta-lactams, including GADD45 and Hsp70. Our results supported by this IDEA award and the Concept Award strongly support our hypothesis that beta-lactams cause tumor DNA damage, which is responsible for their anti-tumor activities. Development of these beta-lactams into potential antitumor drugs is our long-term goal. These studies should provide strong support for proof-of-concept of the potential use of these N-thiolated beta-lactams in breast cancer prevention and treatment.

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10. Di Chen, Samuel C. Falsetti, Michael Frezza, Vesna Milacic, Aslamuzzaman Kazi, Qiuzhi Cindy Cui, Timothy E. Long, Edward Turos, and Q. Ping Dou. Anti-tumor activity of N-thiolated β -lactam antibiotics. *Cancer Letter*, in revision.

APPENDICES

Frezza M, Garay J, Chen D, Cui QZC, Turos E and Dou QP. Induction of tumor cell apoptosis by a novel class of N-thiolated β -lactam antibiotics with structural modifications at N₁ and C₃ of the lactam ring. Intl. J Mol Med., 2008; in press.

Di Chen, Samuel C. Falsetti, Michael Frezza, Vesna Milacic, Aslamuzzaman Kazi, Qiuzhi Cindy Cui, Timothy E. Long, Edward Turos, and Q. Ping Dou. Anti-tumor activity of N-thiolated β -lactam antibiotics. Cancer Letter, in revision.

Other documents.

Curriculum vitae.

Induction of tumor cell apoptosis by a novel class of N-thiolated β -lactam antibiotics with structural modifications at N1 and C3 of the lactam ring

MICHAEL FREZZA¹, JULIO GARAY², DI CHEN¹, CINDY CUI¹, EDWARD TUROS² and Q. PING DOU¹

¹The Prevention Program, Barbara Ann Karmanos Cancer Institute, and Department of Pathology, School of Medicine, Wayne State University, Detroit, MI; ²Department of Chemistry, College of Arts and Sciences, University of South Florida, Tampa, FL, USA

Abstract. The investigation of novel anti-tumor agents that preferentially select for malignant cells with a tolerable toxicity level has been the focus of anti-cancer drug discovery. Our laboratories have previously reported that certain N-alkylthiolated β -lactams had DNA-damaging and apoptosis-inducing activity in various tumor lines but not in nontransformed cells. In the current study, we further delineated the effects of substitutions at C₃ or N₁ of the lactam ring for cell death-inducing capability with close attention paid to a discernible structure-activity relationship (SAR). We found that two β -lactam analogs (JG-5 and JG-19), both containing a branched-chain moiety at C₃ of the lactam ring, exhibit potent apoptosis-inducing activity. Additionally, JG-5 exhibited superior *in vitro* biological activity over JG-19 owing to structural modifications made to substituents at the N₁ and C₃ positions of the lactam ring. Furthermore, the branched β -lactams were able to inhibit growth of mice bearing breast cancer xenografts, associated with induction of DNA damage and apoptosis in tumor tissues. Our results strongly warrant further investigation into these novel β -lactams as potential anti-cancer therapeutics.

Introduction

Apoptosis (programmed cell death) is a natural, physiologic process that is critical in regulating the homeostatic cell number in a tightly controlled manner and deregulation of this process can lead to the pathogenesis of various diseases including cancer (1-3). From a morphological standpoint,

apoptosis is characterized by cell shrinkage, DNA fragmentation and subsequent membrane blebbing and membrane-enclosed apoptotic bodies (3,4). Anti-cancer drugs, including chemotherapy, γ -irradiation and immunotherapy exert their effects predominantly by modulating key elements of the apoptosis cascade (5,6). However, many tumors possess defects in the apoptotic machinery and become resistant to standard cytotoxic regimens, posing serious clinical problems (7-9). Therefore, developing novel anti-cancer therapies that circumvent these limitations is of paramount importance.

The treatment of bacterial infections with β -lactam antibiotics has been the staple of treatment for over a half century. β -lactams such as penicillin represent a potent class of inhibitors of bacterial growth and various moieties have been isolated or synthesized since the discovery of penicillin (10). The discovery of penicillin was the impetus for the introduction and clinical use of various β -lactams, including cephalosporins, penems, carbapenems and monobactams (11). These β -lactam compounds function on bacterial cells by selectively disrupting cross-linking events during the final stage of bacterial cell wall synthesis, leading to subsequent cell lysis (12). These inhibitory properties are specific for bacteria which of course have the peptidoglycan cell wall, which are not found in mammalian tissue and thus do not affect human cell lines. Recently, however, our laboratories discovered a novel class of N-thiolated β -lactams (13,14) that rapidly induce DNA damage, inhibit DNA replication and induce apoptosis in a number of mammalian cell lines including human leukemic T cells, breast, prostate and head and neck (15-17). These surprising findings formed the basis of developing novel β -lactam analogs with various structural modifications in the hope of generating lead candidates that have selective tumor cell apoptosis-inducing activity.

In the current study, we screened a focused library of N-thiolated β -lactam analogs with substitutions made at either N₁ or C₃ of the lactam ring (Fig. 1), with close attention being paid to a possible structure-activity relationship. Our current study shows that manipulation and addition of carbon chain moieties at these two positions correlate directly to their cell death-inducing capability *in vitro*. Two β -lactam analogs, JG-5 and JG-19, were found to exhibit potent anti-proliferative activity tested against human leukemic cell lines

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E-mail: doup@karmanos.org

Key words: β -lactams, structure-activity relationship, apoptosis, DNA damage, tumor cell death

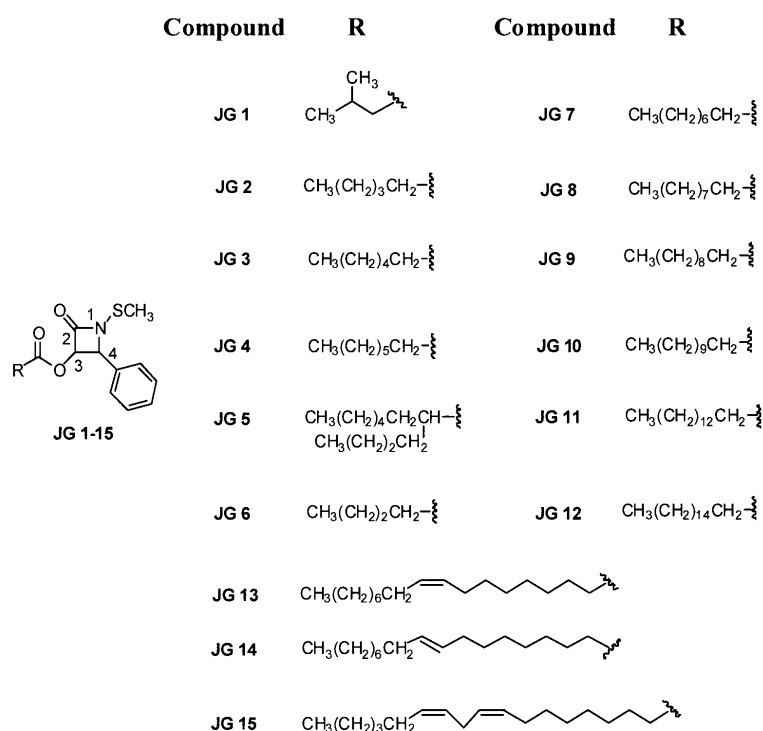
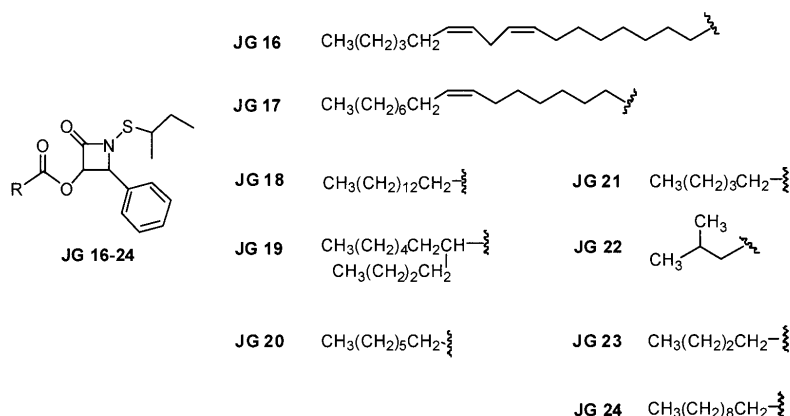
N-Methylthio β -lactam derivatives (JG1-15)N-sec-butylthio β -lactam derivatives (JG16-20)

Figure 1. Structures of 24 representative N-thiolated β -lactams with structural modifications at N_1 and C_3 of the lactam ring. Each β -lactam analog is designated as JG along with numerical representation.

and that this effect directly correlates to its branched moiety at position 3 of the lactam ring structure. Furthermore, this cell death-inducing ability exerted by JG-5 and JG-19 is directly associated with apoptosis as indicated by cleavage of apoptosis-specific poly(ADP-ribose) polymerase (PARP) and staining of apoptotic nuclei. Additionally, the biological effect exhibited *in vitro* was emulated in a human breast cancer xenograft in mice. Our data strongly support the concept of investigating β -lactam antibiotics as a novel approach in the treatment of cancer.

Materials and methods

Materials. The β -lactam analogs were prepared using a procedure described previously (18). Hoechst 33258,

cremophore, dimethylsulfoxide (DMSO) and trypan blue were purchased from Sigma-Aldrich (St. Louis, MO). Apoptag peroxidase *in situ* Apoptosis detection kit was from Chemicon International, Inc. (Temecula, CA). Fetal bovine serum was purchased from Aleken Biologicals (Texarkana, AR). A mixture of penicillin-streptomycin-L-glutamine, RPMI-1640 medium and sodium pyruvate were purchased from Invitrogen (Carlsbad, CA). Monoclonal antibodies to poly(ADP-ribose) polymerase were purchased from Biomol International LP (Plymouth Meeting, PA) and anti-mouse IgG-horseradish peroxidase was obtained from Santa Cruz Biotechnologies (Santa Cruz, CA).

Cell cultures and whole cell extract preparation. Human leukemic Jurkat T, Raji and HL-60 cells were cultured in

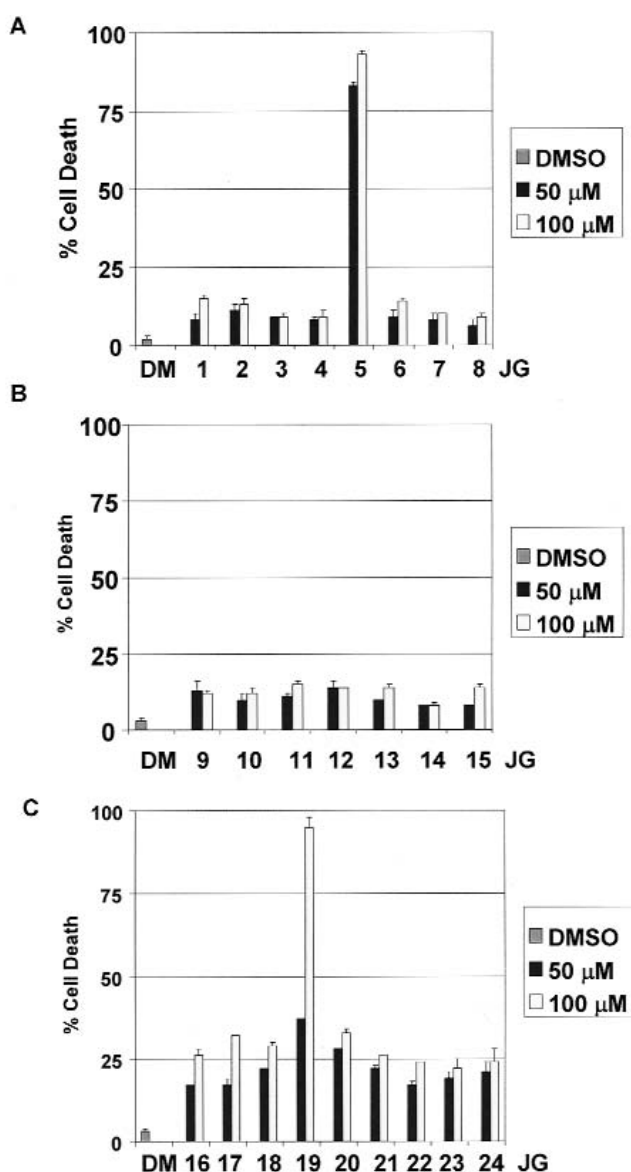


Figure 2. Library screen of β -lactams for more potent analogs. (A and B), Raji leukemic cells were treated with solvent (DMSO), 50 or 100 μ M of each indicated compound for 24 h, followed by trypan blue exclusion assay. (C) HL-60 leukemic cells were treated with solvent (DMSO), 50 or 100 μ M of each indicated analog for 24 h, followed by trypan blue exclusion assay. The numbers given are percentages of nonviable cells to total cells. Standard Deviations are shown with error bars from a mean of three different experiments.

RPMI-1640 medium, supplemented with 10% fetal bovine serum, 100 U/ml penicillin and 10 μ g/ml streptomycin and 1% sodium pyruvate solution (100 mM). All cell lines were maintained in a humidified incubator at 37°C and 5% CO₂. A whole-cell extract was prepared as described previously (19). Briefly, cells were harvested, washed with ice-cold PBS and homogenized in a lysis buffer (50 mM Tris-HCl, pH 7.5, 5 mM EDTA, 150 mM NaCl, 0.5% NP-40) for 25 min at 4°C. Afterwards, the lysates were centrifuged at 12,000 rpm for 15 min at 4°C and the supernatants collected as whole-cell extracts.

Western blot analysis. The cell extracts were separated by SDS-PAGE and transferred to a nitrocellulose membrane.

Western blot analysis was performed using a specific antibody to PARP, followed by visualization using the enhanced chemiluminescence (ECL) reagent (Amersham Biosciences, Piscataway, NJ).

Trypan blue assay. The trypan blue dye exclusion assay was performed by mixing 50 μ l of cell suspension with 20 μ l of 0.4% trypan blue dye before injecting into a hemocytometer and counting. The number of cells that absorbed the dye and those that excluded the dye were counted, from which the percentage of nonviable cell number to total cell number was calculated.

Nuclear staining. A Zeiss Axiovert 25 microscope was used with fluorescence for nuclear morphology with Hoechst 33258 staining as described previously (20).

Human breast tumor xenograft experiments. Five-week-old female athymic nude mice were purchased from Taconic Research Animal Services (Hudson, NY) and housed under pathogen-free conditions according to Wayne State University animal care guidelines. The protocols of animal experiments were reviewed and approved by the Institutional Laboratory Animal Care and Use Committee of Wayne State University. MDA-MB-231 cells were injected subcutaneously (s.c.) at one flank of the mice. The mice were then injected s.c. with either solvent (PBS:cremophor: ethanol: DMSO = 5: 2.7: 1.3: 1) as a control or 10 mg/kg with dose escalation to 20 mg/kg of either β -lactams JG-5 or JG-19 for 29 days. Tumor size was measured every other day using calipers. Tumor volume (V) was determined by the equation: $V = (L \times W^2) \times 0.5$, where L is the length and W is the width of the tumor.

Terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) assay. Tumor tissues were paraffin embedded and stained according to the manufacturer's instructions. Briefly, after deparaffinization and hydration, the tissue was incubated with working strength stop/wash buffer, conjugated with anti-digoxigenin, and then stained with peroxidase substrate. Finally, the tissue was mounted under a glass cover slip in permount and viewed under a microscope.

H&E staining assay. Paraffin-embedded sample slides were deparaffinized and hydrated, and then stained with hematoxylin for 1 min. After rinsing, the slides were then stained with eosin for 1 min, followed by further rinsing, and coverslips were mounted onto slides with permount.

Results

Library screen of N-thiolated β -lactams for cell death-inducing activity. Our laboratories have previously shown that certain β -lactam analogs with structural modifications at N₁ and C₃ of the lactam ring possess apoptosis-inducing ability (16,17). A focused library of β -lactam analogs was synthesized having different alkyl chain lengths and branching properties on the C₃ acyl side chain (Fig. 1), and then each was independently screened for cell death-inducing activity using a trypan blue exclusion assay (Fig. 2). We also examined a second family

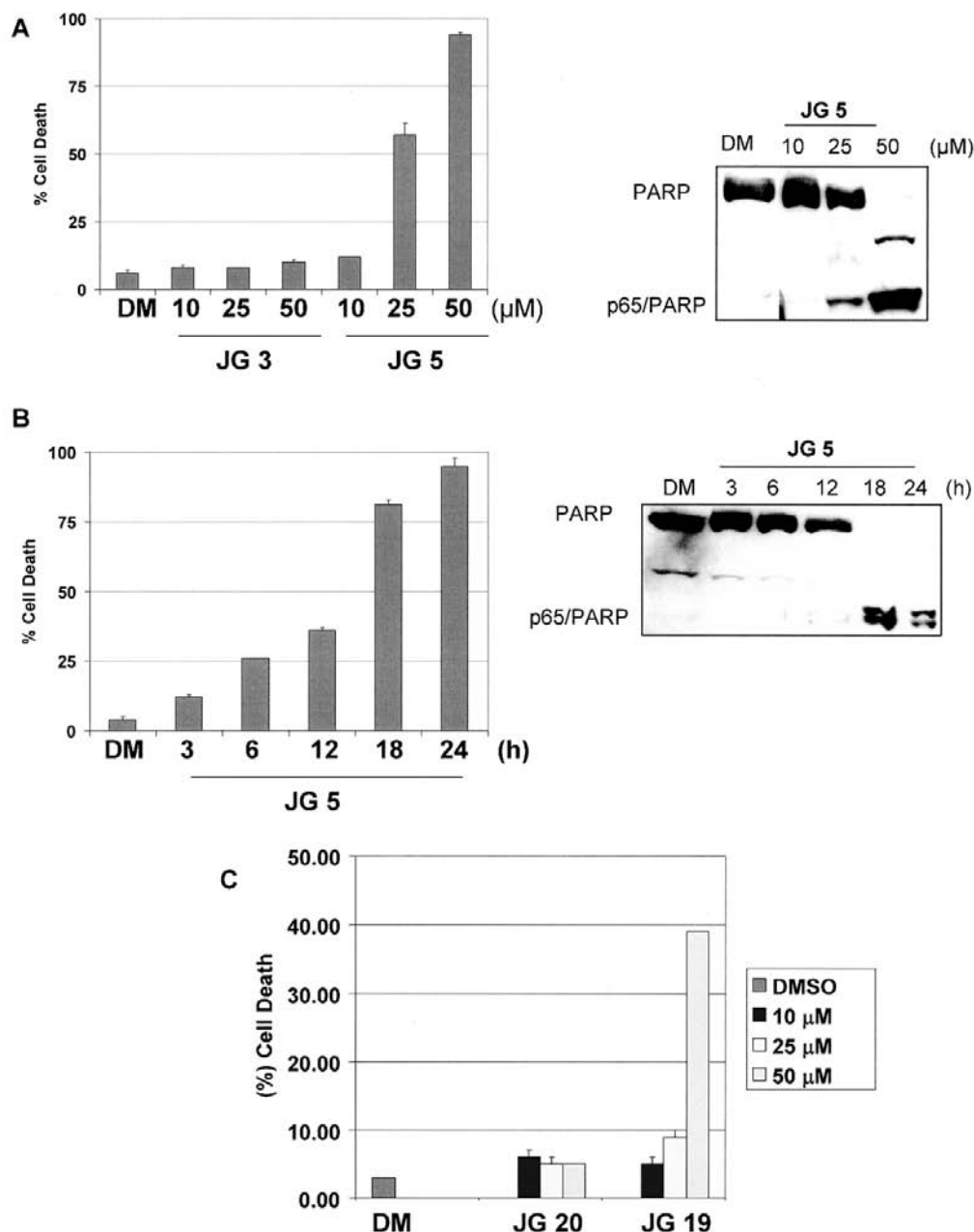


Figure 3. Selective induction of apoptosis by branched lactam analogs (JG-5, JG-19) over their straight chain counterparts (JG-3, JG-20). (A) Jurkat T cells were treated at indicated concentrations for 24 h with either DMSO control, JG-3 or JG-5, followed by measurement of cell death and apoptosis-specific PARP cleavage by Western blot analysis. The intact PARP fragment (116 kDa) and PARP cleavage (p65) are shown. (B) Jurkat T cells were treated with JG-5 at 50 μ M for the indicated hours, followed by measurement of cell death and apoptosis-specific PARP cleavage. (C) Jurkat T cells were treated with DMSO control, JG-20 or JG-19 for 24 h at indicated concentrations and cell death was measured. All results were obtained in three independent experiments.

of N-thiolated β -lactams having a *sec*-butylthio moiety on the N_1 center. The compound screening procedure was undertaken by treating leukemia cells with either 50 or 100 μ M of indicated analog for 24 h, followed by measurement of non-viable cells (Fig. 2). Among the compounds tested, the branched long-alkyl chain derivative JG-5 was able to induce cell death by 83 and 93% at 50 and 100 μ M, respectively (Fig. 2A). Additionally, the corresponding *sec*-butylthio analog JG-19 at these two concentrations was found to induce cell death at 37 and 95%, respectively (Fig. 2C). A common structure-activity relationship that exists between these two lactams JG-5 and JG-19 is the presence of a branched carbon

chain moiety on C_3 of the lactam ring. Additionally, the presence of a double bond within the carbon chain moiety at C_3 of the lactam ring (JG-13 to JG-17) did not appear to significantly affect potency (Fig. 2B and C). Furthermore, lengthening of the carbon chain (without branching) was found to be inversely proportional to its cell killing activity, with increasing straight chain length leading to diminishing activity in this assay (Fig. 2).

Induction of apoptotic cell death by JG-5 and JG-19. We then set out to determine if apoptosis is responsible for the cell-killing activity of the two most active compounds, JG-5 and

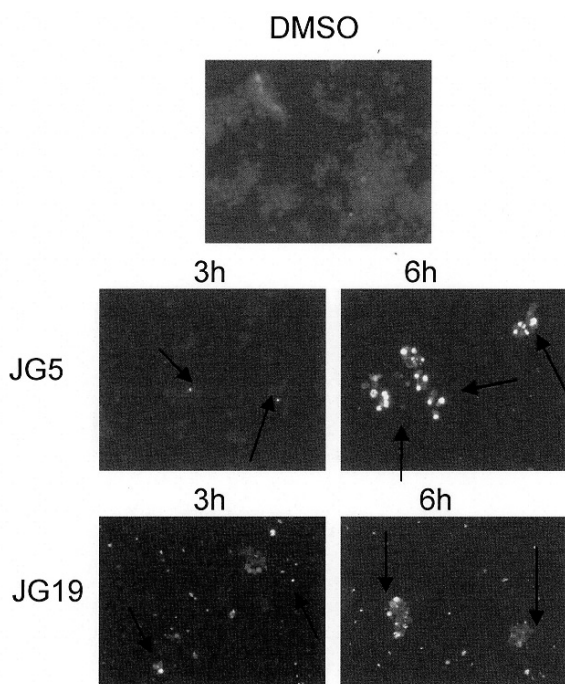


Figure 4. Apoptotic nuclear staining in cells treated with JG-5 and JG-19. To assay nuclear morphology, Raji cells treated with 50 μ M of JG-5 or JG-19 for 3 or 6 h were fixed and stained with Hoechst 33342. The nuclear morphology is visualized by fluorescence microscopy (x40).

JG-19, by measuring for apoptosis-specific PARP cleavage in a Western blot analysis (Fig. 3A and B). Jurkat T cells were first treated at different concentrations of JG-5 and compared to its smaller straight chain counterpart (JG-3) in a trypan blue assay, followed by detection of the cleaved PARP fragment (Fig. 3A). Our results showed that cells treated with JG-5 at 25 and 50 μ M exhibited ~58 and ~95% cell death, respectively. In contrast, <10% cell death was induced by cells treated with JG-3 (Fig. 3A, left). Consistent with the presence of non-viable cells, low levels of PARP cleavage were visible in cells treated at 25 μ M of JG-5. However, after treatment with 50 μ M of JG-5, the full length PARP fragment was fully cleaved into p85 and p65 fragments (Fig. 3A, right). In contrast, no PARP cleavage was detectable in cells treated with JG-3 at the highest concentration tested (data not shown).

In the kinetic experiment, Jurkat T cells were treated with 50 μ M of JG-5 for various points (3–24 h), followed by measurement of nonviable cells and apoptosis-specific PARP cleavage (Fig. 3B). After 18 h and 24 h treatment, cell death was induced at 82 and 95%, compared to 12, 27, and 37% at 3, 6 and 12 h, respectively (Fig. 3B, left). In the same experiment, the p65/PARP cleavage fragment was detected at 18 and 24 h, compared to earlier time points where no detectable levels of p65/PARP were found (Fig. 3B, right). These results suggest that substitution at C₃ of the lactam ring with a branched moiety confers enhanced anti-proliferative activity.

We then compared the potency of the branched acyl analog JG-19 and the straight chain variant JG-20 by measuring cell death in a trypan blue assay. Jurkat T cells were treated with 10–50 μ M of JG-19 or JG-20 for 24 h and measured for non-viable cell population (Fig. 3C). We found that when

cells were treated with 50 μ M of JG-19, cell death was induced ~40%. In comparison, cells treated with JG-19 <50 μ M were mostly viable similar to that obtained with JG-20-treated cells at all concentrations tested. In the same experiment, cells treated with JG-19 and JG-20 were measured for apoptosis-specific PARP cleavage (data not shown). PARP cleavage was detectable only in cell lysates treated with JG-19 at 50 μ M compared to JG-20- and DMSO-treated cells. This suggests that introducing branched alkyl moiety on the sulfur side chain at the same time as having branching in the C₃ acyl residue may partially mitigate the potency compared to JG-5 since our laboratory has previously shown that increasing the number of carbons from one to two on the N-alkylthio group attenuated cell killing activity (16,21).

To further confirm that apoptosis is responsible for the cell killing of JG-5 and JG-19, we investigated the ability of the two branched lactams JG-5 and JG-19 to damage tumor cell DNA. Jurkat T cells were treated with 50 μ M of either JG-5 or JG-19 for 3 or 6 h, followed by Hoechst staining and observation of nuclear morphology (Fig. 4). Cells treated with 50 μ M of JG-5 for 6 h contain significant levels of bright, punctate nuclei consistent with the onset of apoptosis (Fig. 4). In comparison, JG-19-treated cells contained lower levels of brightly stained apoptotic nuclei at 6 h (Fig. 4). Collectively, our results demonstrate tumor cell killing by branched acyloxy residues at C₃ of these β -lactams, with enhanced activity conferred by JG-5.

Cell death-inducing capability of JG-19 and JG-5 is associated with apoptosis induction in breast tumor xenografts. Our *in vitro* results show that JG-19 and JG-5 preferentially induce cell death associated with apoptosis in human leukemia cells. To investigate whether this effect can be emulated *in vivo*, we used mice bearing breast tumor xenografts. MDA-MB-231 cells were implanted s.c. into female nude mice and allowed to grow until the appearance of a palpable tumor (~80 mm³). The mice were then injected s.c. daily with solvent or 10 mg/kg with dose escalation to 20 mg/kg with lactam JG-19 or JG-5 for 29 days. At the end of the trial the mice were sacrificed and their tumor tissue was used for immunostaining assays. Measurement of tumor size showed that tumor growth was inhibited only ~18% with the mouse treated with JG-19 and ~49% in JG-5-treated mouse, compared to solvent control treatment.

To determine if apoptosis was the predominant factor responsible for tumor growth inhibition, prepared tissue samples were used for H&E and TUNEL staining (Fig. 5). Induction of apoptosis was confirmed in tumors treated with JG-5 and JG-19 compared to control-treated tumors by measurable levels of condensed apoptotic nuclei detected in H&E staining (Fig. 5A) and the presence of TUNEL-positive cells (Fig. 5B). JG-19-treated tumor contained low levels of apoptotic markers whereas JG-5-treated tumors contained significantly higher levels of apoptotic markers (Fig. 5), consistent with the effect exhibited *in vitro*. No visible toxicity was observed in either treatment groups as indicated by a stable weight pattern. Collectively, these results clearly show that the branched acyloxy-substituted β -lactams (JG-19 and JG-5) can preferentially induce DNA damage and apoptosis *in vivo*.

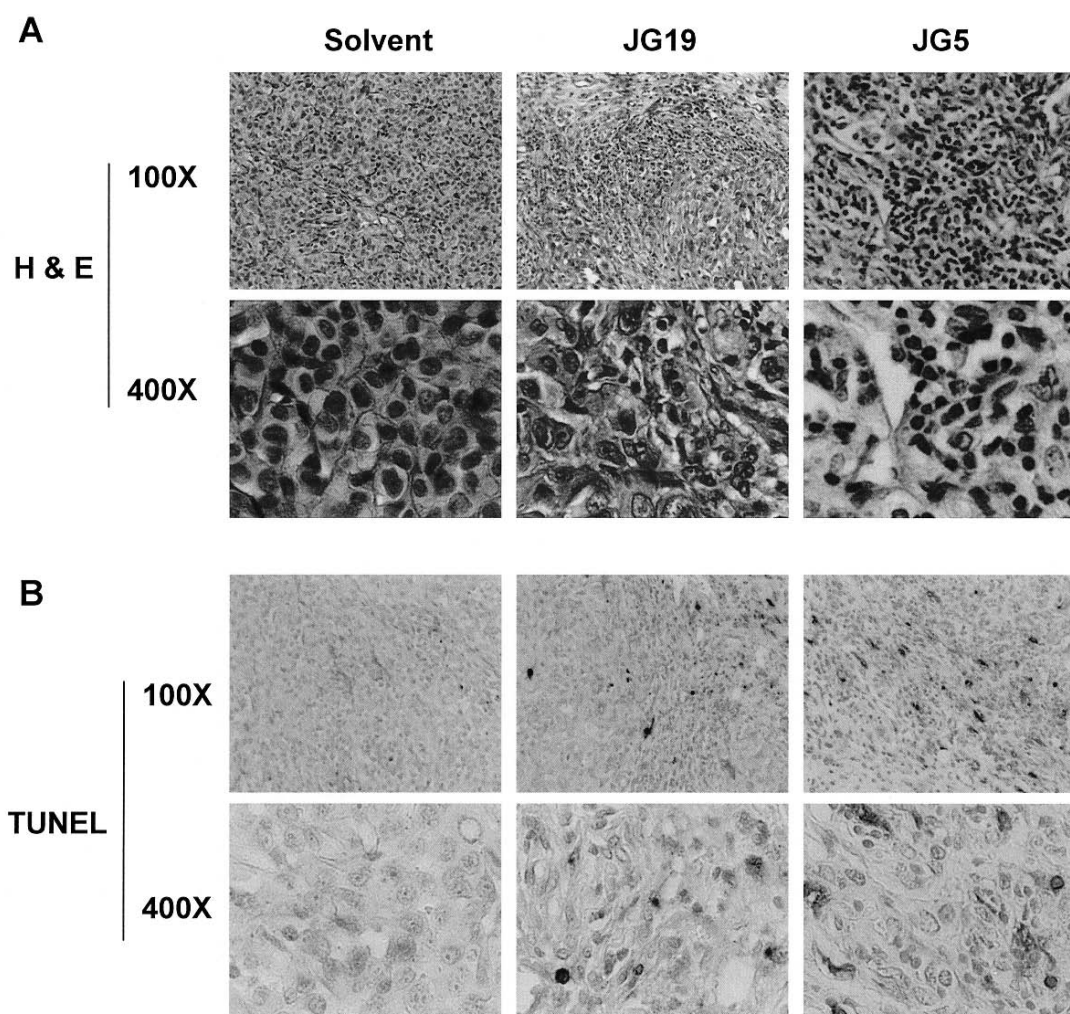


Figure 5. Hematoxylin and eosin (H&E) staining and TUNEL assays using mouse tumor samples. Tumors were collected after a 29-day treatment and the prepared tissue slides were used for immunostaining with H&E (A) and TUNEL staining (B) assays. Apoptotic-condensed nuclei and TUNEL-positive cells were found in tumor tissue from mice treated with JG-5 or JG-19. Magnification: x100 and x400 as indicated.

Discussion

Developing novel anti-cancer agents that preferentially induce apoptosis in tumor cells with an overall favorable toxicity profile is an ongoing endeavor in anti-cancer drug discovery. The established clinical effectiveness of β -lactams with limited toxicity in treating bacterial infections, and the unusual mode of action of N-thiolated β -lactam analogs, prompted our investigation into their effect towards tumor cells. N-thiolated β -lactams represent a novel class of anti-bacterial compounds that significantly inhibit the growth of methicillin-resistant *Staphylococcus aureus* (MRSA) and *Bacillus anthracis* (13,14,22). We have previously shown that certain N-thiolated β -lactams can induce DNA damage in a variety of cell lines, including prostate, breast, and head and neck leading to the induction of apoptosis through S-phase arrest, p38 activation, cytochrome c release and induction of caspase 3 (16,17). Furthermore, we have shown that some of these particular N-thiolated β -lactam modalities can selectively induce apoptosis in human leukemic Jurkat T cells, but not in non-transformed, immortalized human NK cells (15).

In this study, we screened a concentrated library of N-alkylthio β -lactam analogs to generate additional lead

candidates for further preclinical development. Our laboratory has previously shown that substitution at each position of the lactam ring plays a unique role in conferring efficacy. The N-methylthio group is imperative for imparting biological activity and that eliminating this moiety or adding more carbon chains in the alkyl residue results in a substantial decrease of potency (21). The carbonyl at position 2 of the lactam ring is characterized as the backbone of the lactam molecule and therefore cannot be manipulated without losing its overall activity. We have previously reported on the effects of additions/manipulations at position 3 of the lactam ring (16). Our current study is a refined extension of the structure-activity relationship between various substituent groups at positions 1 and 3 of the lactam ring, and how this influences the overall efficacy of the molecule.

The results of our screen generated two potent analogs that are directly related to the nature of substituent groups at position N₁ and C₃ of the 4-membered lactam ring. JG-19 and JG-5 were selected from a group of 24 representative β -lactam analogs that possess a branched carbon chain moiety at position 3 of the lactam ring. Furthermore, JG-19 possesses a *sec*-butylthio group at the nitrogen position 1 of the lactam ring. Our results demonstrate that branched β -lactam

analogs confer a selective advantage in inducing cell death. Additionally, this cell death-inducing ability is directly associated with apoptosis as indicated by apoptosis-specific PARP cleavage and the appearance of bright, punctuate apoptotic nuclei (Figs. 3 and 4). This effect was demonstrated in a time- and concentration-dependent manner. However, JG-19 demonstrated a much lower level of potency as indicated by the fact that cell death was only induced ~40% at 50 μ M and generated only low levels of PARP cleavage (Fig. 3C and data not shown). This observation is consistent with previous work in our laboratory that suggests that increasing the number of carbons within the N-alkylthiol group decreases potency (17). This may also preclude tight binding to its cellular target within the apoptotic cascade requiring higher concentrations to reach a similar effect as JG-5.

An important aspect of our study is to investigate the apoptosis-inducing affect *in vivo* and whether this effect is associated with reduction of tumor burden in mice bearing breast tumor xenografts. Therefore, we tested the effects of JG-5 and JG-19 in mice bearing human MDA-MB-231 xenografts. Our data showed that mice treated with JG-5 reduced tumor growth by (~49%) and JG19 (~18%) with no visible levels of toxicity. Immunostaining by H&E and TUNEL (Fig. 5) confirmed that reduction of tumor burden is directly related to apoptosis.

While the molecular mechanism of N-alkylthio β -lactams are not fully characterized and still under investigation, our results and previous studies suggest that these unusual β -lactams possess selective apoptosis-inducing activity. Further investigation into their potential for use as anti-cancer drugs is warranted.

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Anti-tumor activity of N-thiolated β -lactam antibiotics

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Abstract

An ongoing strategy for cancer treatment is selective induction of apoptosis in cancer over normal cells. *N*-thiolated beta-lactams were recently found to inhibit proliferation and induce cell cycle arrest and apoptosis in human tumor cells. Using microarray analysis, we show here that β -lactam L-1 treatment up-regulated mRNA levels of numerous DNA-protective genes, including HSP70 and GADD45 β . We also demonstrate that β -lactam L-1 treatment caused a significant inhibition of tumor growth in mice bearing human breast cancer xenografts. This was associated with the accumulation of GADD45 β and Hsp70 proteins and apoptosis induction. Taken together, these results strongly suggest that β -lactam L-1 holds significant potential to be developed as a novel anti-cancer drug.

1. Introduction

Apoptosis, or programmed cell death, is an evolutionarily conserved cellular suicide program essential for the development of multi-cellular organisms [1]. By removing unwanted cells, apoptosis plays a vital role in development, tissue homeostasis, and defense against viral infections and mutations. In many human diseases, the genes controlling the apoptotic process are suppressed, over-expressed or altered by mutations [2]. Importantly, the vast majority of human cancers are associated with mutations in various apoptotic checkpoint proteins, or tumor suppressor genes. A preponderance of epidemiological and molecular evidence suggests that these mutations play a critical role in enabling the progression of malignancy through suppression of the apoptotic process [3].

The three fundamental steps of apoptosis are initiation, commitment, and execution [4]. Several apoptotic stimuli, such as irreparable DNA damage, have been shown to activate the cellular caspase enzymatic cascade pathway, which is a central part of apoptosis. It is believed that the subsequent proteolytic cleavage of a variety of intracellular substrates, such as poly(ADP-ribose) polymerase (PARP) and the retinoblastoma (Rb) protein, by effector caspases leads to the terminal hallmarks of apoptosis, such as nuclear blebbing [5-8].

Selective activation of apoptotic pathways in cancer cells, but not in normal ones, is currently being pursued as a novel strategy for the cancer treatment. However, it has been shown that standard cytotoxic chemotherapeutic drugs induce apoptosis not only in malignant but in normal cells as well, while more aggressive cancer cells become resistant. This narrow therapeutic window often necessitates aggressive dosing regimens which are frequently discontinued due to toxic side-effects. Therefore, it is essential to develop targeted therapies that minimize toxic side effects by specifically targeting and destroying tumors without harming non-malignant tissue.

Small synthetic molecules with apoptosis-inducing ability have potential to be developed into novel chemotherapeutic drugs. These molecules can be easily synthesized and structurally manipulated for selective development [9]. For more than 60 years, β -lactam antibiotics have played an essential role in treating bacterial infections [10, 11]. Since these β -lactam drugs selectively disrupt the formation of bacterial cell walls, eukaryotic cells are not affected, making β -lactam therapy safe for the patients. Recently, a new class of *N*-thiolated β -lactams was found to inhibit growth in methicillin-resistant *Staphylococcus aureus* [12-15]. Subsequent work has demonstrated that these *N*-thiolated

β -lactams are capable of inducing apoptosis in tumor cells, making them good candidates for anti-cancer drugs [9].

We have previously reported that β -lactam L-1 (Fig. 1A), the most potent of the *N*-thiolated β -lactams tested, induces DNA damage, inhibits DNA replication and activates the apoptosis in cultured human tumor cells in a time- and concentration-dependent manner [9]. We have also demonstrated that L-1 selectively induces apoptosis in leukemic Jurkat T over normal immortalized YT cells and that L-1 induces apoptosis in several solid human tumor cell lines [9]. Although inhibition of p38 MAP kinase was identified as a central event in the anti-tumor activity of β -lactam L-1 [9], the main cellular target of the β -lactam remains unknown.

In an attempt to identify the molecular targets for β -lactam L-1, we analyzed the global transcriptional response of Jurkat T cells to β -lactam L-1 treatment. Specifically, we used an AffyMetrix human mRNA microarray analysis and showed, for the first time, that L-1 up-regulates mRNA expression of a number of genes encoding DNA-interacting proteins, such as Growth Arrest and DNA Damage Inducible Beta (GADD45 β) and Heat Shock Protein-70 (HSP-70). This increased mRNA expression was detected as early as after 2 hours of L-1 treatment and was further potentiated after 24 hours of treatment. We also show that L-1 inhibits proliferation and induces apoptosis in the highly metastatic breast cancer cell line, MDA-MB-231, in a concentration-dependent manner. Using subcutaneous xenografts of human MDA-MB-231 implanted in nude mice, we confirmed the ability of L-1 (20 and 30 mg/kg) to damage tumoral DNA, induce apoptosis and inhibit tumor growth *in vivo* in a dose responsive manner. Importantly, the ability of L-1

to induce DNA damage and apoptosis *in vivo* was correlated with potent accumulation of GADD45 β and HSP-70 proteins.

2. Materials and methods

2.1. Materials

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Trypan blue and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (St. Louis, MO). Polyclonal antibodies to actin, monoclonal antibodies to GADD45 β and HSP70, and anti-goat and anti-mouse IgG-horseradish peroxidase were obtained from Santa Cruz Biotechnologies (Santa Cruz, CA). Mouse monoclonal antibody against human poly (ADP-ribose) polymerase (PARP) was purchased from BIOMOL International LP (Plymouth Meeting, PA).

2.2. Synthesis of β -Lactam 1

The N-thiolated β -lactam L-1 was synthesized using a procedure described previously [12, 15].

2.3. Cell culture, protein extraction, and Western blot assay

Human Jurkat T cells were cultured in RPMI 1640 medium, supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 μ g/ml streptomycin. Human breast cancer MDA-MB-231 cells were grown in DMEM/F12 (1:1) supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 μ g/ml streptomycin. Both cell lines

were maintained at 37°C in a humidified incubator with an atmosphere of 5% CO₂. A whole cell extract was prepared as previously described [16]. For Western blot analysis, the cell or tissue extracts were separated by SDS-PAGE and transferred to a nitrocellulose membrane, followed by visualization using the enhanced chemiluminescence (ECL) reagent (Amersham Biosciences, Piscataway, NJ).

2.4. Trypan blue assay

The trypan blue dye exclusion assay was performed by mixing 20 µl of cell suspension with 20 µl of 0.4% trypan blue dye before injecting into a hemocytometer and counting. The number of cells that absorbed the dye and those that exclude the dye were counted, from which the percentage of nonviable cell number to total cell number was calculated.

2.5. RNA extraction and purification

RNA was extracted from Jurkat T-cell culture following removal of media by centrifugation. Cells were lysed in RNazole reagent and RNA was separated from the cell lysate following manufacturer's recommendations. The extracted RNA was further purified using the Rneasy purification kit (Qiagen) according to manufacturer's specifications and quantified using absorbance readings at 260 nm and 280 nm on a Beckman DU640B spectrophotometer. Purity was checked by comparison of 28S and 18S ribosomal RNA bands for incubated (1 hour at 55°C) and non-incubated samples run on a 1% polyacrylamide gel. Bands were visualized using ethidium bromide stain and UV light detection.

2.6. Microarray and data analysis

Jurkat T cells were treated with 60 μ M/L of L-1 for 2 or 12 hours, harvested for total RNA and subjected to microarray study using the human gene array chips (U-133A) from Affymetrix. These chips contained 22,215 probe sets, which target known and suspected genes as well as a number of suspected splice variants. The data were analyzed by using an Affymetrix Microarray 5.1 (MAS 5.0) software. The MAS 5.0 software uses a statistical algorithm to assess increases or decreases in mRNA abundance in a direct comparison between two samples. The algorithm is calculated from the behavior of 11 different oligonucleotide probes designed to detect the same genes. Probe sets that yield a change p-value less than 0.005 are identified as changed (increased or decreased).

2.7. Cellular proliferation assay

MTT assay was used to determine the effect of β -lactam L-1 on overall proliferation of breast cancer MDA-MB-231 cells. Cells were plated in a 96-well plate and grown to 70-80% confluency, followed by addition of L-1 at indicated concentrations for 24 h. MTT (1 mg/ml) in PBS was then added to wells and incubated at 37°C for 4 hours to allow for complete cleavage of the tetrazolium salt by metabolically active cells. Next, MTT was removed and 100 μ l of DMSO was added, followed by colorimetric analysis using a multilabel plate reader at 560 nm (Victor³; Perkin Elmer). Absorbance values plotted are the mean from triplicate experiments.

2.8. Human breast tumor xenograft experiments

Female athymic nude mice aged 5 weeks were purchased from Taconic Research Animal Services (Hudson, NY) and housed in accordance with protocols approved by the Institutional Laboratory Animal Care and Use Committee of Wayne State University. Human breast cancer MDA-MB-231 cells (5×10^6) suspended in 0.1 ml of serum-free RPMI 1640 were inoculated subcutaneously (*s.c.*) in both flanks of each mouse. When tumors reached sizes of $\sim 150 \text{ mm}^3$ in average, the mice were randomly grouped ($n = 7$) and treated by daily *s.c.* injection with either 20 or 30 mg/kg of L-1 or the solvent (PBS containing 1% DMSO). Tumor size was measured every other day using calipers and their volumes were calculated according to a standard formula: $\text{width}^2 \times \text{length} / 2$. Mice were sacrificed after 29 days of treatment when control tumors reached $\sim 900 \text{ mm}^3$. The tumors were collected and the tumor tissues were used for different assays for measuring proteasome inhibition and cell death.

2.9. Terminal nucleotidyl transferase-mediated nick end labeling (TUNEL) and Hematoxylin and Eosin assays using tumor tissue samples

TUNEL assay using *in situ* apoptosis detection kit was performed as described previously [17]. Hematoxylin and Eosin (H & E) staining in tumor tissue was performed following manufacturer's protocols. Western blot analysis using animal tumor samples was performed similarly as described above using cultured cancer cells.

2.10. Statistical analysis

To evaluate the difference between the treatment and solvent control, the Student's *t* test was applied. Variance analysis of ANOVA (Statistical Computation DASC software) was used to test differences among multiple independent groups *in vivo* study. The statistic difference was indicated as $P < 0.05$ and significant difference as $P < 0.01$.

3. Results

3.1. *GADD45* β and *HSP-70* are two of the main targets for L-1 in Jurkat T cells

We have previously reported that *N*-thiolated β -lactams have the ability to induce DNA damage and apoptosis selectively in transformed and malignant, but not in non-transformed cells [18]. However, the mechanisms for DNA-damaging and apoptosis-inducing effects, as well as for the selectivity of these β -lactams, are still unknown. In an attempt to answer these questions, we used microarray assay to analyze the gene expression profile in Jurkat T cells following L-1 treatment. To select time points for microarray analysis, we were specifically interested in the cells that have already sustained DNA damage but have not undergone apoptosis, in comparison to the cells in which the apoptosis has already started. A time course experiment in which Jurkat T cells were treated with 60 $\mu\text{mol/L}$ of L-1 was conducted and the cells were analyzed for cell viability (Trypan blue dye exclusion assay), DNA damage (TUNEL assay), and PARP cleavage (Western blot). We found that 2 hour treatment of Jurkat T cells with 60 $\mu\text{mol/L}$ of L-1 resulted in 22% of nonviable cells (Fig. 1B) and induced strong DNA damage (76.3% TUNEL-positive cells) (Fig. 1C), but not apoptosis as confirmed by the absence of cleaved PARP protein (Fig. 1D). However, after 12 hour of treatment, 56% Trypan blue-positive

cells (Fig. 1B) and 96.8% TUNEL-positive cells (Fig. 1C) were detected, together with the apoptosis induction confirmed by PARP cleavage (Fig. 1D).

Therefore, we isolated RNA from Jurkat T cells treated for 2 and 12 hours, converted it to cDNA and analyzed by the Affymetrix U133A chips and Affymetrix Microarray 5.0 (MAS 5.0) software. By analyzing over 22,000 genes, we found that expression of many genes was increased by more than 2-fold, following just 2 hours of treatment with L-1. A sample of 13 genes was selected for this study (Table 1). Among them, a number of genes encoding DNA-interacting proteins was significantly affected, including growth arrest and DNA damage inducible gene-45 (GADD45), inhibitor of DNA binding 2 (ID2) and zinc finger 38 (ZF38). The fact that induction of these genes preceded apoptosis suggests that up-regulation of DNA-interacting proteins is an important component of the cellular response to L-1 treatment. An almost 44-fold increase in GADD45 β mRNA expression was detected after 2 hour treatment (Table 1), which was associated with a significant increase in the level of GADD45 β protein after 12 hours of treatment with 60 μ mol/L of β -lactam 1 (Fig. 1E).

The largest transcriptional increase was observed in multiple isoforms of Hsp70 mRNA (e.g., isoform 1A, 67-fold increase after a 2 hour treatment, Table 1). This significant up-regulation of Hsp70 gene transcription was followed by a consequent increase in Hsp70 protein level, starting at 6 hours of treatment, as showed by Western blot assay (Fig. 1D).

3.2. L-1 inhibits proliferation and induces apoptosis in human breast cancer MDA-MB-231 cells

Our results demonstrated that L-1 has the ability to induce cell death in leukemia Jurkat T cells (Fig. 1). We next investigated whether the same effect could be observed in solid tumor cell lines, such as human breast cancer MDA-MB-231 cells. We first tested the anti-proliferative potency of L-1, by plating the MDA-MB-231 cells in 96-well plate and treating them with various concentrations of L-1 for 24 hours, followed by an MTT assay. We found that L-1 inhibits proliferation of MDA-MB-231 cells in a dose-dependent manner (Fig. 2A); at 25 $\mu\text{mol/L}$ concentration L-1 inhibited cell proliferation by approximately 41% (p value < 0.01) whereas at 75 $\mu\text{mol/L}$ concentration it induced 80% inhibition (p value < 0.01). Inhibition of MDA-MB-231 cell proliferation by L-1 was associated with a dose-dependent PARP cleavage (Fig. 2B), which is a hallmark of apoptosis. These data show that the ability of L-1 to inhibit cell proliferation is associated with apoptosis induction not only in leukemia Jurkat T-cells but also in breast cancer MDA-MB-231. After L-1 treatment, the protein levels of GADD45 β and Hsp70 in MDA-MB-231 cells were also increased in a dose-dependent manner (Fig. 2B).

3.3. The growth-inhibitory and apoptosis-inducing effects of L-1 in human breast tumor xenografts

After demonstrating that L-1 could efficiently inhibit proliferation and induce apoptosis in cultured MDA-MB-231 cells, we investigated its anti-tumor activity *in vivo*. Human breast cancer MDA-MB-231 cells (5×10^6) were implanted *s.c.* in five-week-old female athymic nude mice. Upon reaching a palpable size ($\sim 150 \text{ mm}^3$), the mice were daily *s.c.* injected with either 20 or 30 mg/kg of L-1, or solvent control for 29 days. At the end of the trial, the measurement of tumor size showed that tumor growth was inhibited by

35 and 62 % in the mice treated with 20 mg/kg and 30 mg/kg of L-1, respectively (Fig. 3A). The statistical analysis showed there were significant differences among the three groups (the solvent, low dose and high dose of L-1), and between each L-1 treatment and solvent groups as well.

We have shown that treatment of cultured Jurkat T and MDA-MB-231 cells with L-1 increased the levels of GADD45 β and Hsp70 proteins (Figs. 1-2). To investigate if a similar effect could be observed *in vivo*, we extracted proteins from the tumor remnants and used them for Western blot analysis. We found that induction of GADD45 β protein occurred after both 20 and 30 mg/kg treatments whereas induction of Hsp70 protein was most pronounced at 30 mg/kg treatment (Fig. 3B).

To determine whether DNA damage and apoptosis are responsible for the observed anti-tumor activity of the *N*-thiolated β -lactams, TUNEL assay and H & E staining were performed using formalin-fixed tumor tissue. DNA strand breaks indicated by TUNEL positivity were observed in tumors from animals treated with the β -lactam, but not control (Fig. 3C). Another apoptotic feature, condensed nuclei, was shown by Hematoxylin and Eosin staining in the tumors from mice treated with the β -lactam (Fig. 3C). Taken together, these data show that the L-1 has anti-breast tumor ability, associated with its ability to induce DNA damage and apoptosis in breast tumor tissues *in vivo*.

4. Discussion

Since many currently used anti-cancer therapies are toxic and eventually cause resistance to the treatment, the search for non-toxic yet effective alternative continues. It is generally believed that an important property of a potential anticancer drug is its ability

to induce apoptosis, preferably in a highly selective manner in cancer over normal cells. It has been known that antibiotic therapies typically use the unique molecular targets of microbes, which makes them safe for the patient. β -lactam antibiotics as a class are powerful and potent inhibitors of bacterial growth [10] and interestingly, one group called *N*-thiolated β -lactams, possesses anti-proliferative activity in human tumor cells [9]. Since they were also observed to have little to no effect on normal cells [18], their anti-cancer potential and expected lack of toxicity classified them as excellent candidates for anti-cancer drug development.

Our previous work with *N*-thiolated β -lactams demonstrated that these compounds act through DNA damage that subsequently induces S-phase arrest, and apoptosis [9]. Apoptosis induced by these compounds is caspase-dependent and associated with cytochrome c release [9], and is selectively induced in cancerous over normal cells [18]. We have also published results showing that the increase in p38 phosphorylation is critical for β -lactam-induced apoptosis, since abrogation of pp38 activity with a specific inhibitor (PD169316) leads to tumor cell survival [9]. However, the question remains how p38 gets activated by β -lactam treatment.

Here, using an Affymetrix microarray we analyzed the response of Jurkat T cells to β -lactam L-1 treatment. We screened over 22,000 gene transcripts in the cells with L-1-induced DNA damage without apoptotic program started, and in the cells in which apoptosis was already triggered. We found that β -lactam L-1 treatment up-regulated mRNA level of DNA-protective genes, such as HSP70 and GADD45 family (particularly one of its members - GADD45 β). The increased transcription resulted in an increased protein level, as confirmed by Western blot analysis. Moreover, inhibition of HSP70 with

genestein significantly decreased cell membrane permeability (data not shown), revealing a critical role of HSP70 in a cellular response to β -lactam L-1 treatment. Since HSP70 protein has a number of interacting partners, what exactly follows the HSP70 over-expression remains unclear. It has been known that the major heat-inducible HSP70 is a potent survival protein that confers cytoprotection against numerous death-inducing stimuli and increases the tumorigenicity of rodent cells. However, it has also been demonstrated that HSP70 stimulates the polymerase β DNA base excision repair enzyme [19]. Therefore, it is possible that in a response to DNA damage induced by β -lactam L-1 treatment, HSP70 protein translocates to the nucleus and gets involved in DNA damage repair by interacting with DNA, either directly or through binding partners. This possibility, however, needs further investigation.

A GADD45 family protein was shown to be involved in activation of G₂/M cell cycle arrest following DNA damage, and in the regulation of S phase checkpoint [20]. Moreover, GADD45 family proteins function as specific activators of MTK1, a MAPK kinase kinase upstream in the p38 pathway [21], and induce apoptosis [22]. It has been suggested that specific binding of GADD45 α to p38 is a potential mechanism by which GADD45 α has a direct role in p38 activation by Ha-Ras [23]. It was also reported that ectopic expression of GADD45 β in AML12 cells is sufficient to activate p38 and to trigger apoptotic cell death, whereas antisense inhibition of *Gadd45 β* expression blocks TGF- β -dependent p38 activation and apoptosis [20]. Furthermore, TGF- β was shown to activate p38 and induce apoptosis in mouse primary hepatocytes from wild-type mice, but not from *Gadd45b* knockout mice [20]. All of these findings suggest that GADD45 β participates in TGF- β -induced apoptosis by acting upstream of p38 activation. Since we

have previously identified p38 MAP kinase as a central player in β -lactam-induced apoptosis, our results strongly suggest that GADD45 β is a primary target for β -lactam L-1, which after induction by the treatment activates p38, thereby inducing apoptosis.

The most important aspects in our study were to investigate whether β -lactam L-1 is active *in vivo* and to verify its molecular targets. Therefore, we tested the effects of β -lactam L-1 in mice bearing human MDA-MB-231 xenografts. Our data showed that treatment with β -lactam L-1 caused a significant inhibition of MDA-MB-231 tumor growth in nude mice (Fig. 3A). Importantly, the anti-tumor activity of β -lactam L-1 was associated with the accumulation of GADD45 β and HSP70 proteins (Fig. 3B), and induction of apoptosis (Fig. 3C). Taken together, our current study strongly suggests that *N*-methylthio β -lactams, such as L-1, offers significant potential to be developed as novel anti-cancer drugs.

Acknowledgments

The authors thank Department of Defense Breast Cancer Research Program Awards (W81XWH-04-1-0688 and DAMD17-03-1-0175 to Q.P.D.) for support of this research. We also acknowledge the Karmanos Cancer Institute Pathology Core Facility for assisting in TUNEL and immunohistochemistry assays and Moffitt Core Facility for assisting in microarray analysis.

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Figure legends

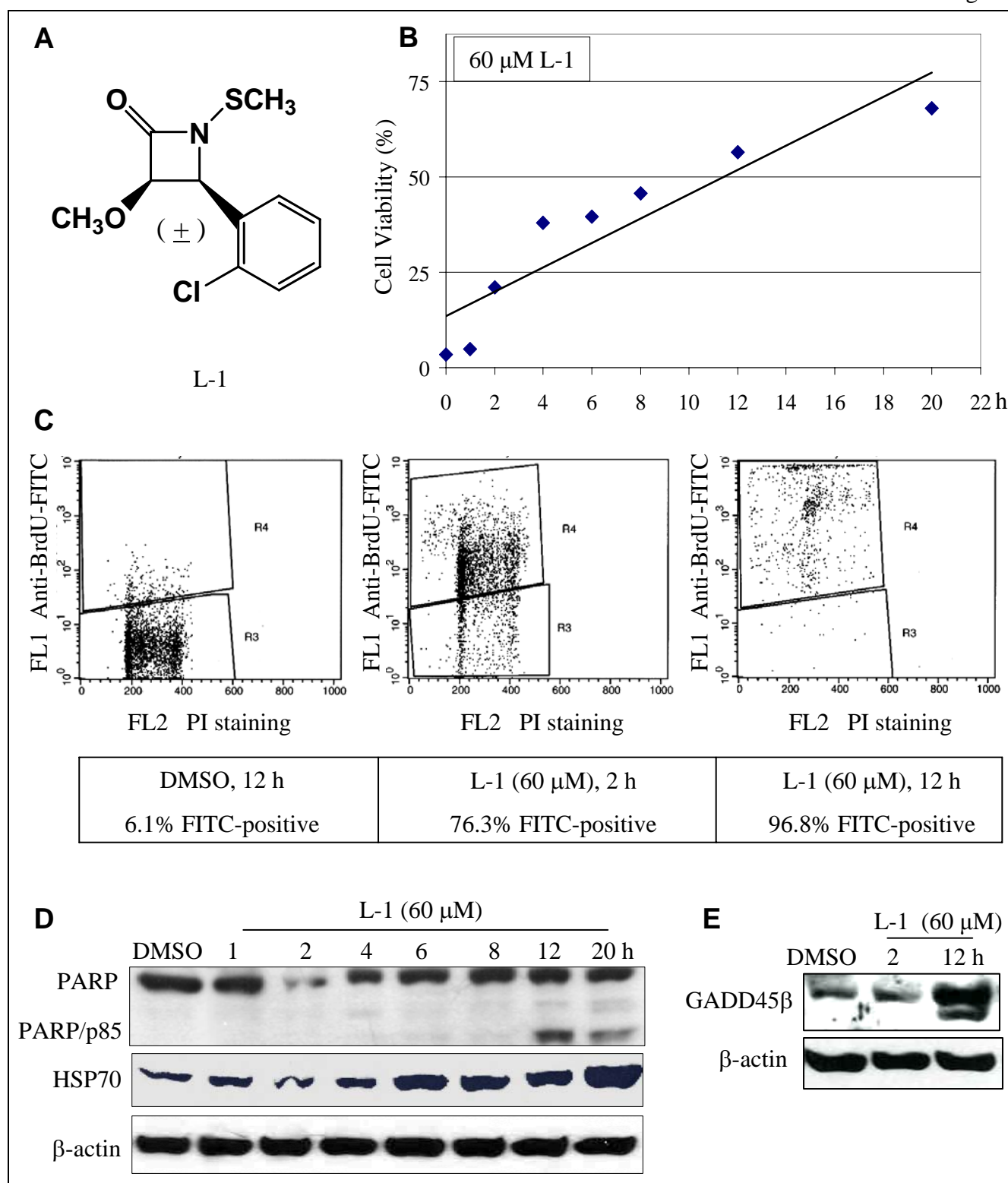
Figure 1. Induction of apoptosis in human leukemia Jurkat T cells by *N*-methylthio β -lactam L-1 (L-1) treatment. (A) Chemical structure of L-1. (B) Jurkat T cells were treated with 60 μ mol/L L-1 for indicated time points, followed by Trypan blue dye exclusion assay. (C) Jurkat T cells were treated for 2 and 12 hours with 60 μ mol/L of L-1, followed by TUNEL assay. DMSO was used as a control. (D) Western blot analysis of Jurkat T cells treated as indicated, using antibodies against PARP, HSP70 and β -actin. DMSO was used as a control. (E) Western blot analysis of Jurkat T cells treated for 2 and 12 hours, using antibodies against GADD45 β and β -actin.

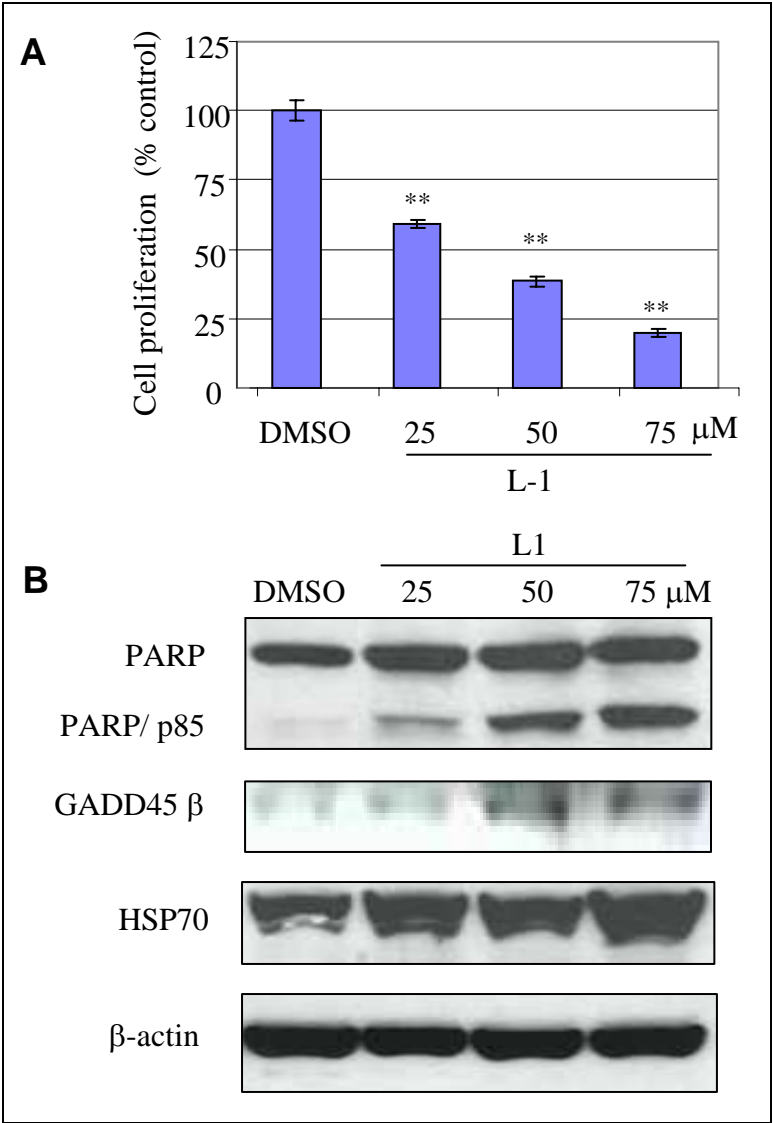
Figure 2. Inhibition of cell proliferation and induction of apoptosis in MDA-MB-231 cancer cells. (A) Highly metastatic and malignant breast cancer MDA-MB-231 cells were treated for 24 hours with different concentrations of L-1 (25, 50, and 75 μ mol/L). After 24 hours, medium was removed, and cells were treated with MTT solution as described in Materials and Methods. ** $P < 0.01$; Bars, SD, mean of three experiments. (B) Western analysis of samples prepared from MDA-MB-231 cells treated for 24 hours as indicated.

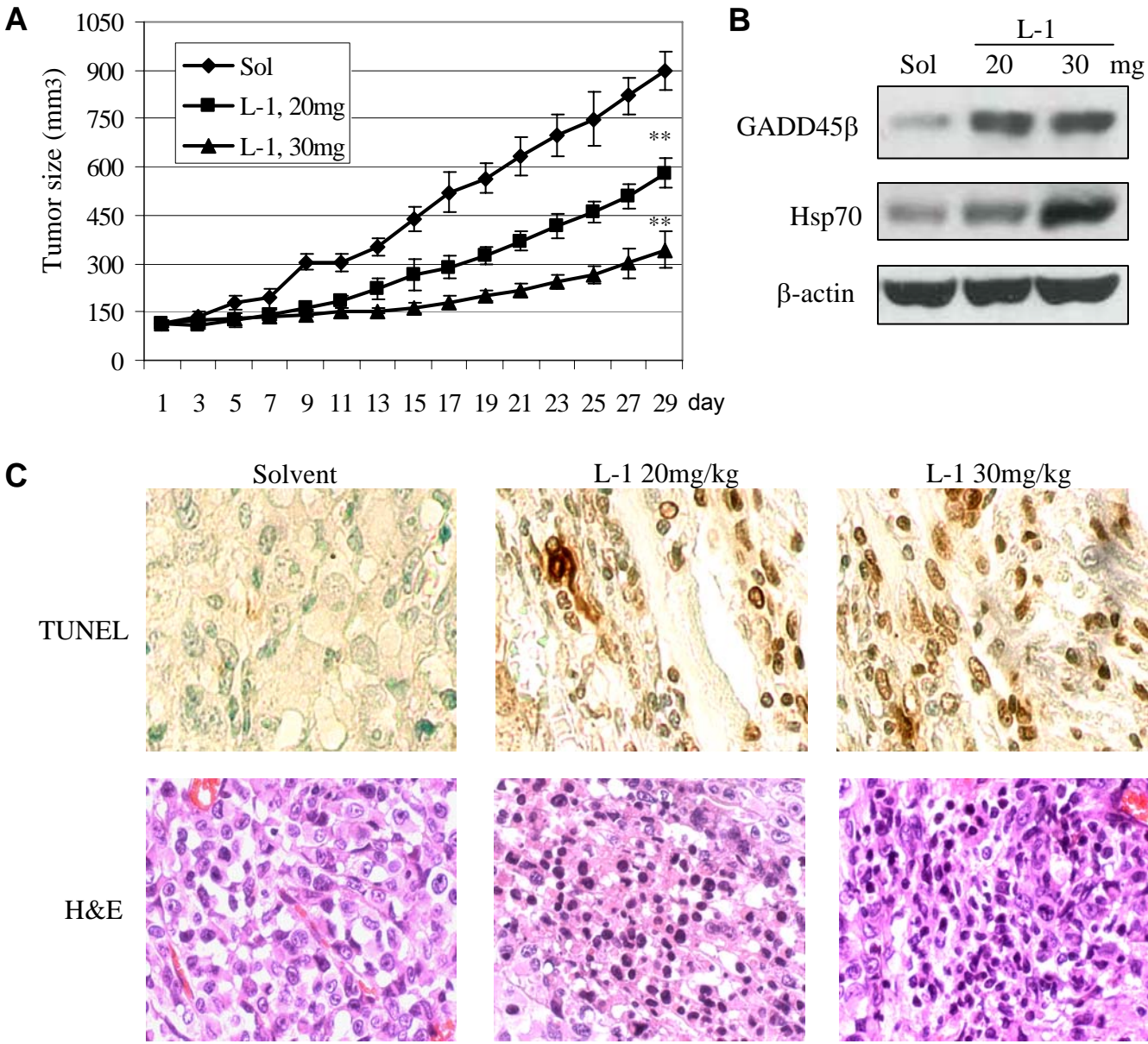
Figure 3. The anti-tumor activity of L-1 is associated with up-regulation of GADD45 β and HSP70 proteins and induction of apoptosis *in vivo*. Female nude mice bearing MDA-MB-231 tumors were treated with either control solvent (Sol) or L-1 at 20 or 30 mg/kg/d to day 29. (A) Inhibition of MDA-MB-231 tumor growth by L-1. Points, mean tumor volume in each experimental group containing seven mice; SD. **, $P < 0.01$. (B) Effects of L-1 at the end point of the experiment. Tumors were collected after 29 days of treatment, and the prepared tissues were analyzed by Western blotting, using antibodies against GADD45 β , HSP70 and β -actin. (C) TUNEL assay and H & E staining using mice tumor samples.

Table 1. A list of genes up-regulated in Jurkat T cells after the treatment with 60 $\mu\text{mol/L}$ of β -lactam L-1 for 2 and 12 hours.

Probe set ID number	Fold change 2h 12h		Accession number and Gene name
207614_s	3.3	5.2	gb:NM_006352.1; Zinc finger 38
222074_at	3.3	4.1	gb:AW614435; Urophorinogen decarboxylase
201566_x	5.1	7.9	gb:D13891.1; Id-2H inhibitor of DNA binding 2
213988_s	6.5	17.1	gb:BE971383; Spermidinespermine N1-Acetyl Transferase
202912_at	3.2	11.9	gb:NM_001124.1; Adrenomedullin (ADM)
201565_s	5.3	18.9	gb:NM_002166.1; Inhibitor of DNA Binding 2 (ID2)
201739_at	7.1	20.1	gb:NM_005627; Serum Glucocorticoid Regulated Kinase (SGK)
202388_at	3.2	22.9	gb:NM_002923.1; Regulator of G-protein Signaling 2, 24KD (RGS2)
207574_s	9.2	56.7	gb:NM_015675.1; Growth Arrest and DNA Damage Inducible Beta (GADD45)
209304_x	43.7	254.2	gb:AF_087853.1; Growth Arrest and DNA Damage Inducible Beta (GADD45B)
202581_at	6.2	59.9	gb:NM_005346.2; HSP 70 Protein 1B
213418_at	7.8	410.2	gb:NM_002155.1; HSP 70 Protein 6
200800_s	66.9	3505.2	gb:NM_005346.2; HSP 70 Protein 1A







ANNUAL REPORT REVIEW
USAMRMC FY02 BREAST CANCER RESEARCH PROGRAM

Grant/Contract/MIPR No.: DAMD17-03-1-0175

Principal Investigator: Q. Ping Dou, Ph.D.

Institution: Wayne State University
Detroit, Michigan

Report Title: Synthetic beta-Lactam Antibiotics as a Selective Breast Cancer Cell Apoptosis Inducer: Significance in Breast Cancer Prevention and Treatment

Report Type: Annual (Fifth, Revised)

Award Mechanism: Idea

Date of Report: March 2007

Reporting Period: 1 March 2006 – 28 February 2007

SUMMARY REVIEW: Control of apoptotic mechanisms, through apoptosis-inducing small molecules, may be used in the prevention and treatment of cancers, including breast cancer. Beta-lactam antibiotic family members have played a role in treating bacterial infections for over 60 years. The Principal Investigator (PI) discovered new members of this family of nontoxic small molecules, termed N-thiolated beta-lactams, that inhibit bacterial growth in drug-resistant bacterial strains and are anti-proliferative and apoptosis-inducing toward human cancer cells. The PI proposed to characterize the mechanism of action of N-thiolated beta-lactams.

Lactam 1, a member of the N-thiolated beta-lactam family of compounds, was demonstrated to have apoptotic activity restricted to human cancer cells and did not affect nontransformed, immortalized cells. A screen of related compounds showed that lactam 12 had the highest apoptosis-inducing activity, which was found to be a result of increased DNA damage. Lactam 12 induced apoptosis in human breast, prostate, and head-and-neck-derived cancer cells but not in nontransformed and parental normal fibroblast cells. In vitro assay data demonstrated that lactam 1 and lactam 12 both inhibited colony formation of human prostate cells, suggesting that this family of compounds may be useful anticancer agents.

To evaluate the effects of structural changes, the PI evaluated compounds with substitutions of the O-methyl moiety on C3 and stereochemistry changes. Lactam 18, where C3 was substituted with an acryl ester group, was shown to inhibit the cellular proliferation of human premalignant and malignant breast, leukemic, and simian virus 40-transformed fibroblast cells. An analysis of many compounds demonstrated an inverse relationship between the size of the moiety on C3 and

the anti-proliferative activity. In addition, it was found that the stereochemistry of the beta-lactams played an important role in their potency.

The PI reports the effects of N-thiolated beta-lactams on bacteria. This family of compounds was shown to selectively inhibit the growth of Staphylococcus bacteria and a variety of Bacillus species. Additional findings suggest that these lactams react rapidly within the bacterial cells with coenzyme A through in vivo transfer of the N-thiol group.

During the past year, the PI has also constructed a second library of more polar and more water-soluble lactam derivatives. Results indicated that several beta-lactams were potent cell-death inducers in leukemia HL60 and Raji cell lines. Interestingly, two of these beta lactams, JG19 and JG20, could induce PARP cleavage. The PI also found that the L-47 lactam had the greatest potency to induce PARP cleavage in MDA-MD-231 cells. The L-1 lactam was found to induce cell death by 50% in Jurket cells and was also found to increase the expression of heat shock protein 70. The PI also investigated whether genes displayed an increase in expression upon treatment with L-1. Results showed that several DNA interacting proteins, such as GADD45 and zinc finger 38, were affected.

FORMAT/EDITORIAL ISSUES: This revised fifth annual report generally conforms to USAMRMC reporting requirements. The PI is reminded that the key research accomplishments section should be a bulleted list of the major research findings of the past year.

CONTRACTUAL ISSUES: Information provided in this revised fifth annual report supports the following:

Task 1	Months 1-18	Completed
Task 2	Months 19-36	In progress

TECHNICAL ISSUES: None.

Dou, Ping

From: Scassero, Shannyn M Ms USAMRAA [shannyn.scassero@us.army.mil]
Sent: Friday, February 01, 2008 8:39 AM
To: Dou, Ping; manderson@med.wayne.edu; info@karmanos.org
Subject: DAMD17-03-1-0175; Increase in Funds (UNCLASSIFIED)
Importance: High
Follow Up Flag: Follow up
Due By: Thursday, February 07, 2008 12:00 PM
Flag Status: Flagged

Classification: **UNCLASSIFIED**

Caveats: NONE

All,

The original Awardee (this award was transferred from H. Lee Moffitt Cancer Center to Wayne State on 17 MAR 04) for the referenced award incorrectly determined the amount of funding expended on their portion of this award. An additional \$3,443.38 has been returned to the Government for this award. I am in the process of drafting the modification to provide Wayne State University the funds, but need to request a budget and justification for the funds and a determination by Wayne State University as to whether the funds are needed for award performance.

Due to the short time until award expiration, I request this information be provided no later than 7 FEB 08, so that the Government has time to issue the modification.

If any questions, please contact me for assistance.

Thank you so much,
Shannyn M. Scassero
Contract Specialist
USAMRAA
(P) 301-619-2640

Classification: **UNCLASSIFIED**

Caveats: NONE

Dou, Ping

From: Anderson, Michael [MAnderso@med.wayne.edu]
Sent: Thursday, February 07, 2008 11:10 AM
To: shannyn.scassero@us.army.mil
Cc: Wade, Valerie; Dou, Ping (KCI)
Subject: FW: DAMD17-03-1-0175; Increase in Funds (UNCLASSIFIED)

Importance: High

Attachments: Budgut and Jus.doc



Budgut and Jus.doc
(27 KB)

Ms. Scassero;

As discussed this morning, attached please find the budget and justification request for the remaining funds that were relinquished and transferred to Wayne State University from H. Lee Moffitt Cancer Center on behalf of our P.I. Q. Ping Dou.

Please accept this email as institutional concurrence and approval for the above mentioned request. If there are any questions/comments, please do not hesitate to contact either the PI, or our office.

Thank you again for your consideration regarding this matter.

Sincerely,

Mike A.

Michael A. Anderson, Grants and Contracts Officer Sponsored Programs Administration, Wayne State University School of Medicine / University Health Center
4201 St. Antoine / 9D UHC
Detroit, Michigan 48201
(313) 577-9554 (phone)
(313) 577-1348 (fax)
email: manderso@med.wayne.edu

-----Original Message-----

From: Dou, Ping [mailto:doup@karmanos.org]
Sent: Friday, February 01, 2008 9:46 AM
To: Anderson, Michael
Subject: FW: DAMD17-03-1-0175; Increase in Funds (UNCLASSIFIED)
Importance: High

FYI

Q. Ping Dou, Ph.D.
Leader, Prevention Program
Barbara Ann Karmanos Cancer Institute, and Professor, Department of Pathology School of Medicine Wayne State University
540.1 HWCRC
4100 John R Road
Detroit, MI 48201-2013
Tel: 313-576-8301 (Office); 313-576-8299 (Adm. Assistant); 313-576-8264, -8248, -8249 (Lab)
Fax: 313-576-8307 (Office); 313-576-8928 (Adm. Assistant)
E-mail: doup@karmanos.org

From: Dou, Ping
Sent: Friday, February 01, 2008 9:44 AM
To: Wade, Valerie
Cc: 'manderson@med.wayne.edu'; info@karmanos.org; Dou, Ping
Subject: FW: DAMD17-03-1-0175; Increase in Funds (UNCLASSIFIED)
Importance: High

Hi Valerie,

Per request, please find the included budget and justification for the increased fund (\$3,443.38).

Please let me know if you need any additional information.

Thanks!

Q. Ping Dou, Ph.D.
Leader, Prevention Program
Barbara Ann Karmanos Cancer Institute, and Professor, Department of Pathology School of
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E-mail: doup@karmanos.org

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Importance: High

Classification: UNCLASSIFIED
Caveats: NONE

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Thank you so much,
Shannyn M. Scassero
Contract Specialist

USAMRAA
(P) 301-619-2640

Classification: UNCLASSIFIED
Caveats: NONE

CURRICULUM VITAE

Q. Ping Dou, Ph.D.

Date of Preparation: March 15, 2008

Signature: _____

OFFICE ADDRESS

The Prevention Program
Barbara Ann Karmanos Cancer Institute, and
Department of Pathology
Wayne State University School of Medicine
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HOME ADDRESS

886 University Place
Grosse Pointe, MI 48230
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EDUCATION:

B.S. in Chemistry, Shandong University, Jinan, Shandong, People's Republic of China, 1981
Ph.D. in Chemistry, Rutgers University, Piscataway, NJ (Mentor: Kuang Yu Chen), 1988

TRAINING:

Postdoctoral Research Fellow in Molecular Biology and Pharmacology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA (Mentor: Arthur B. Pardee), 1988-1992

FACULTY APPOINTMENTS:

Assistant Professor, Department of Pharmacology, University of Pittsburgh School of Medicine, Pittsburgh, PA, 1993-1998

Assistant Professor, Biochemistry and Molecular Genetics Graduate Training Program, Interdisciplinary Biomedical Graduate Program, University of Pittsburgh School of Medicine, Pittsburgh, PA, 1997-1998

Member, Experimental Therapeutic Program, University of Pittsburgh Cancer Institute, Pittsburgh, PA, 1993-1998

Member in Residence, Drug Discovery Program, H. Lee Moffitt Cancer Center & Research Institute, Tampa, Florida, 1998-2003

Associate Professor, Department of Biochemistry and Molecular Biology, University of South Florida College of Medicine, Tampa, Florida, 1998-2003

Associate Professor, Department of Interdisciplinary Oncology, University of South Florida College of Medicine, Tampa, Florida, 2000-2003

Member, the Institute for Biomolecular Science, University of South Florida, Tampa, Florida, 1998-2003

Assistant Program Leader and Scientific Member, Population Studies and Prevention Program, Barbara Ann Karmanos Cancer Institute, Detroit, MI, 2003-present

Leader and Scientific Member, Prevention Program, Barbara Ann Karmanos Cancer Institute, Detroit, MI, 2003-present

Full Professor (with Tenure), Department of Pathology, Wayne State University School of Medicine, Detroit, MI, 2003-present

Full Professor, Cancer Biology Program, Barbara Ann Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, MI, 2003-present

Full Member, Gene Regulation and Genetics Research Program, Institute of Environmental Health Sciences, Wayne State University, Detroit, MI, 2004-present

Member, Scientific Leadership Council (SLC), Barbara Ann Karmanos Cancer Institute, Detroit, MI, 2004-present

Nano Faculty Researcher, the NanoSciences Institute, Wayne State University, Detroit, MI, 2005-present

Honorary Professor in the Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, China, 2006-

Honorary Professor in the Institution of Environmental Sciences, Shandong University, Jinan, Shandong, China, 2006-

Honorary Professor, Wuhan Botanical Garden/ Wuhan Institute of Botany, The Chinese Academy of Sciences, Wuhan, Hubei, China, 2006-

Honorary Professor, Guangzhou Medical College, Guangzhou, China, 2007-

Honorary Professor, The Fourth Wuxi People's Hospital affiliated to Suzhou University, Wuxi, Jiangsu, China, 2007-

Visiting Professor, Ocean University of China, Qingdao, China, 2007-

MAJOR PROFESSIONAL SOCIETIES:

American Association for Cancer Research, Inc.

American Association for the Advancement of Science

American Society for Biochemistry and Molecular Biology

American Society for Pharmacology and Experimental Therapeutics

Society of Chinese Bioscientists in America

International Society for Study of Comparative Oncology, Inc.

American Chemistry Society

New York Academy of Sciences (1995-1998)

HONORS AND AWARDS:

Summer Research Prize in recognition of outstanding accomplishments in research. Rutgers University, 1988

Biochemical Research Support Grant Award. Dana-Farber Cancer Institute, 1991

Barr Program Small Grant Award. Dana-Farber Cancer Institute, 1992

Co-Discussion Leader, University of Pittsburgh Cancer Institute Scientific Retreat, 1995

NIH Director James A. Shannon Award, 1 R55 AG/OD13300-01, 1995-1997

NIH FIRST Award, R29 AG13300-05, 1996-2001

A Predoctoral Trainingship in Breast Cancer Biology and Therapy from the United States Army Medical Research, Development, Acquisitions, and Logistics Command (to Cheryl L. Fattman), 1997-1999

The Best Poster Presentation (An B *et al.*), Scientific Retreat, Department of Pharmacology, University of Pittsburgh School of Medicine, 1997

Chairman for Session of Clinical Oncology/Apoptosis. 2nd World Congress on Advances in Oncology, Athens, Greece, 16-18 October, 1997

Award for the Best Abstract, 2nd World Congress on Advances in Oncology, Athens, Greece, 16-18 October, 1997

Cheryl L. Fattman, Ph.D. Graduation with Honor from University of Pittsburgh (mentor: Q. Ping Dou), 1999

Moffitt's Cancer Center Director's Award (for the article published by Li B and Dou QP in Proc. Natl. Acad. Sci. USA, 2000; 97: 3850-3855). Moffitt Cancer Center & Research Institute, 2000

An AACR-AFLAF Scholar-in Training Award (\$400 to Aslam Kazi/ mentor: QP Dou), for a selected poster (Abstract #788, Kazi A, Smith DM, Zhong Q and Dou QP. Down Regulation of Bcl-X_L Protein Expression by Green Tea Polyphenols Is Associated with Prostate Cancer Cell Apoptosis. AACR-NCI-EORTC. Molecular Targets and Cancer therapeutics, Miami Beach, FL, October 29-November 2, 2001

Abstract was chosen as one of the selected few for News Briefing (Abstract #788, Kazi A, Smith DM, Zhong Q and Dou QP. Down Regulation of Bcl-X_L Protein Expression by Green Tea Polyphenols Is Associated with Prostate Cancer Cell Apoptosis. AACR-NCI-EORTC. Molecular Targets and Cancer therapeutics, Miami Beach, FL, October 29-November 2, 2001

Nominee of Team Award (Cancer Control Program), 2002

Kenyon G. Daniel, Ph.D. a Winner of the 2004 Outstanding Dissertation Award from University of South Florida (Major Professor: Q. Ping Dou), 2004

Invited Visiting Professor in the Department of Urology at the University of California San Francisco and San Francisco VA Medical Center (April 28, 2005), with seminar presentation, "Searching for novel polyphenol proteasome inhibitors for cancer prevention and treatment"

Training Grant (Kristin Landis; Mentor: Q. Ping Dou). Wayne State University, 2005-2007

First Place, Oral Presentation (Kristin Landis; Mentor: Q. Ping Dou). Wayne State University School of Medicine, Graduate Student Research Day, September 22, 2005. "Molecular Studies for the Regulation of Green Tea Polyphenol Biological Function by the Polymorphic Gene Product Catechol-O-Methyltransferase".

Awardee (Kristin Landis; Mentor: Q. Ping Dou) for the Honors Recognition Program for Wayne State University School of Medicine, Wayne State University, 2005

Invited Visiting Professor in the Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, China (April 19-20, 2006), with seminar presentation, "Cancer Prevention and the Role of Environmental Factors". Received Honorary Professor Title.

Invited Visiting Professor in the Institution of Environmental Sciences, Shandong University, Jinan, Shandong, China (April 24-25, 2006), with seminar presentations, "Nutrient-Gene-Environment Interactions and Molecular Prevention of Cancer" and "Discovery of Novel Small Molecules from Nature and Laboratories for Cancer Therapies"

The First Place Poster Award (Huanjie Yang, Di Chen, Qiuzhi Cindy Cui, Xiao Yuan, and Q. Ping Dou). The 6th Annual Symposium, Michigan Prostate Research Colloquium, Basic and Clinical Advances in Prostate Cancer Research, University of Michigan Medical School, Towsley Center, Dow Auditorium, Ann Arbor, MI 48109, May 6, 2006. "Celastrol, A Triterpene Extracted From The Chinese Thunder Of God Vine, Is A Potent Proteasome Inhibitor And Suppresses Human Prostate Cancer Growth In Nude Mice".

Honorary Professorship, Wuhan Botanical Garden/ Wuhan Institute of Botany, The Chinese Academy of Sciences, Wuhan, Hubei, China (October, 2006), with seminar presentation, "Discovery of Novel Small Molecules for Cancer Therapies".

Nominee of the 2007 AACR Landon Prize for Basic Research.

First Place, Oral Presentation (Kristin Landis; Mentor: Q. Ping Dou). Wayne State University School of Medicine, Graduate Student Research Day, September 21, 2006. "A Novel Pro-drug of Green Tea Catechin (-)-EGCG as a Potential Anti-Breast Cancer Agent".

Co-Session Chairman. The 3rd International Symposium on Persistent Toxic Substances, Beijing, China, October 22-25, 2006

Winner of Karmanos Cancer Center Director's Award (for the article published by Yang HJ, Chen D, Cui QC, Yuan X, and Dou QP in Cancer Research 66, 4758-4765, 2006). Karmanos Cancer Institute, 2006

Publication highlighted by Karmanos Cancer Center Director (published by Reddy GPV, Barrack ER, Dou QP, Menon M, Pelley R, Sarkar FH, Sheng S. in Journal of Cellular Biology 2006;98:1407-1423). Karmanos Cancer Institute, 2006

Awardee (Kristin Landis; Mentor: Q. Ping Dou) for the Honors Recognition Program for Wayne State University School of Medicine, Wayne State University, 2006

Invited Speaker. Disulfiram. Molecular Therapeutics of Cancer, Gordon Research Conference, Colby-Sawyer College, New London, New Hampshire, July 22-27, 2007.

Publication highlighted by Karmanos Cancer Center Director (published by Chen D, Cui QC, Yang H, Barrea RA, Sarkar FH, Sheng S, Yan B, Reddy GPV and Dou QP in Cancer Research 2007; 67: 1636-1644). Karmanos Cancer Institute, 2007

Nominee (Michael Frezza; Mentor: Q. Ping Dou) for 57th annual Lindau Meeting with Nobel Laureates, 2007, Lindau, Germany

Invited Speaker. Green Tea Polyphenols as a Natural Tumor Cell Proteasome Inhibitor. The 3rd International Symposium on Functional Foods, PLANT POLYPHENOS, "What would life be without plant polyphenols?" Hong Kong Polytechnic University, Hung Hom, Kowloon, Hong Kong SAR, P.R. China, April 03-05 2008

Invited member of the International Advisory Committee for the International Conference on Nutrition and Cancer, Antalya, Turkey, May 20-23, 2008

Nominee of the 2007 Wayne State University School of Medicine Research Excellence Award

Invited Visiting Professor in The Fourth Wuxi People's Hospital affiliated to Suzhou University, Wuxi, Jiangsu, China (July 1, 2007). Received Honorary Professor Title.

Invited Visiting Professor in Ocean University of China, Qingdao (July 9, 2007).

Invited Visiting Professor in Guangzhou Medical College, Guangzhou, China (July 19, 2007). Received Honorary Professor Title.

First Place, Oral Presentation (Vesna Milacic; Mentor: Q. Ping Dou). Wayne State University School of Medicine, Graduate Student Research Day, September 22, 2007. "Potential use of gold(III) dithiocarbamates as proteasome inhibitors and apoptosis inducers for breast cancer treatment".

Awardee (Vesna Milacic; Mentor: Q. Ping Dou) for the Honors Recognition Program for Wayne State University School of Medicine, Wayne State University, 2007

SERVICE:

Professional Service Activity

Tours for University of Pittsburgh Cancer Institute

Tours for Drug Discovery Program Moffitt Cancer Center & Research Institute

Advisor for Project LINK (Leaders In New Knowledge) Students

Advisor for Moffitt Summer Interns

Advisor for Undergraduate Student Honor's Thesis Research

Member, the Scientific Advisory Board, Torrey Pharmaceuticals, LLC, San Diego, CA, 2000-present

Graduate student recruitment faculty for Cancer Biology Program Karmanos Cancer Institute and Wayne State University, April 3, 2004, April 2, 2005

Presentation to Cancer Biology Program Candidates, Karmanos Cancer Institute and Wayne State University, March 25, 2006 (Kristin Landis and Vesna Minic from Dr. Dou's laboratory)

Preferred Consultant, MEDACorp, Boston, Massachusetts, 2004-present

Council Member, Gerson Lehrman Group, New York, NY, 2004-present

Speaker (Kristin Landis from the laboratory of Q. Ping Dou), "Friends Raising Funds" program, March 5th, 2006, the Powerhouse Gym, West Bloomfield, MI.

Invited Speaker, "Green Tea and Cancer Prevention", "The Day of Wellness" Program, September 16, 2006, the Grosse Pointe War Memorial, Grosse Pointe, MI.

Moderator, Population Studies and Prevention Program Scientific Retreat, Lung Cancer session, Karmanos Cancer Institute and Wayne State University, September 15, 2006

Invited Speaker, Green Tea and its Role in Cancer Prevention. Dow and Towsley Foundation Visit, Karmanos Cancer Institute Research, Detroit, MI, October 11, 2006

Presentation to Cancer Biology Program Candidates, Karmanos Cancer Institute and Wayne State University, April 5, 2008 (Vesna Minic from Dr. Dou's laboratory)

Invited member of the International Advisory Committee for the International Conference on Nutrition and Cancer, Antalya, Turkey, May 19-23, 2008

Journal/Editorial Activity

Editorial Board Memberships

Invited member of the Editorial Board of the *Oncology Reports*, 1996-present

Invited member of the Editorial Board of *Frontiers In Bioscience*, 1997-present

Invited member of the Editorial Board of *LifeXY* (Currently *International Archives of Biosciences*), 2001-present

Invited panel evaluator of *Current Drugs*, 2001-present

Invited member of *The Science Advisory Board*, 2002-present

Invited managing editor of *Frontiers In Bioscience*, 2003-present

Invited managing editor of *Frontiers In Bioscience* for a special issue of "Potential Molecular Targets for Chemoprevention", 2004-present

Invited member of the Editorial Board of ***Current Pharmaceutical Design*** [Impact Factor: 5.27 (2006 SCI Journal Citation Reports)], 2007-present

Invited Executive Guest Editor of ***Current Pharmaceutical Design*** [Impact Factor: 5.27 (2006 SCI Journal Citation Reports)] for a special issue of "**Pharmaceutical design of novel anticancer agents: a lesson from nature**", 2007-present

Reviewer for Journal Manuscripts

Proceedings of National Academy of Sciences USA

FASEB J

Oncogene

Chemistry & Biology

Medicinal Research Reviews (an impact factor of 7.9)

Cancer Research

Clinical Cancer Research

Molecular Cancer Therapeutics

Cell Death & differentiation
Molecular Pharmacology
Journal of Pharmacology & Experimental Therapeutics
Drug Discovery Today (an impact factor of 7.152, 2006)
Exp Cell Res.
J Cellular Physiology
Microbes and Infection
Leukemia
Neoplasia
Cancer Letters
FEBS Letters
Carcinogenesis
International J. Cancer
International J. Oncology
International J. Biochem & Cell Biol
Breast Cancer Research and Treatment
PLoS ONE
J. Cell. Biochemistry
Biochemical Pharmacology
Current Pharmaceutical Design [Impact Factor: 5.27 (2006 SCI Journal Citation Reports)]
BMC Cancer
Life Sciences
Leukemia and Lymphoma
Cancer Chemotherapy and Pharmacology
Lipids
European Journal of Medicinal Chemistry
European Journal of Cancer
Endocrine
Apoptosis
Anti-Cancer Drugs
Chemical Research in Toxicology
Journal of Andrology
J. Dermatological Science
Arch Biochem Biophys
Journal of Pharmacy and Pharmacology
Head & Neck
Journal of Agriculture and Food Chemistry
Obesity Research
Molecular Nutrition and Food Research
Journal of Natural Products
Acta Biochimica et Biophysica Sinica
Natural Immunity
Molecular Biology Reports
Gene Therapy
Cellular and Molecular Life Sciences
Expert Opinion on Investigational Drugs
Expert Opinion on Drug Metabolism & Toxicology
Expert Review of Anticancer Therapy
Expert Review of Proteomics
Evidence-Based Integrative Medicine

Targeted Proteins Database (TPdb)
Life XY (Currently *International Archives of Biosciences*)
The Pittsburgh Undergraduate Review

Reviewer for Grant Applications

Competitive Medical Research Fund (Ad Hoc Reviewer), University of Pittsburgh School of Medicine, 1996

Competitive Medical Research Fund, University of Pittsburgh School of Medicine, 1997

Central Research Development Fund, University of Pittsburgh, 1997

National Science Foundation, 1998

Merit Review Subcommittee for Oncology, the Veterans Affairs Medical Research Programs, US Department of Veterans Affairs, 1999-2005

Member, Cancer Study Section, The Tobacco-Related Disease Research Program, University of California, San Francisco, CA, 2004-present

Invited Reviewer, Biotechnology and Biological Sciences Research Council Research Grant, United Kingdom, 2004, 2006

Invited Proposal Reviewer, The Kentucky Science and Engineering Foundation's R&D Excellence Program, Lexington, KY, 2004, 2005

Invited Reviewer, the Strategic Research Initiative Awards, Barbara Ann Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, MI, 2005

Invited Reviewer, the Seed Money Grants, Barbara Ann Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, MI, 2006

Invited Reviewer, the European Commission's Research Program on "Combating Cancer" - Apoptosis, Brussels, Belgium, 2006

Invited Proposal Reviewer, Ohio Cancer Research Associations, Columbus, Ohio, 2008

Other Professional Related Service

Faculty reviewer for applications to University of Pittsburgh and University of Pittsburgh Cancer Institute

Faculty reviewer for applications to Moffitt Cancer Center & Research Institute and University of South Florida

Faculty reviewer for applications to Karmanos Cancer Institute and Wayne State University (2003-)

Faculty reviewer for Deputy Director/Associate Center Director Candidates to Karmanos Cancer Institute and Wayne State University (2004)

Faculty reviewer for Lambert – Webber Endowed Chair, Division Chief, Hematology and Oncology, Department of Internal Medicine, Wayne State University, Program Leader, Developmental Therapeutics, Karmanos Cancer Institute (2005)

Faculty reviewer for Director of Translational Research Laboratory, Karmanos Cancer Institute, Wayne State University (2007)

National and International Boards and Committees

Ad Hoc Reviewer, National Science Foundation, 1998

Ad Hoc Reviewer, Merit Review Subcommittee for Oncology, the Veterans Affairs Medical Research Programs, US Department of Veterans Affairs, 1999-2001

Member, Department of Veterans Affairs (VA) Medical Research Service Merit Review Subcommittee for Oncology, 2001-2005

Member, the Scientific Advisory Board, Torrey Pharmaceuticals, LLC, San Diego, CA, 2000-present

Council Member, Gerson Lehrman Group's Council of Healthcare Advisors, New York, NY, 2004-present

Member, Cancer Study Section, The Tobacco-Related Disease Research Program, University of California, San Francisco, CA, 2004-present

Invited Reviewer, Biotechnology and Biological Sciences Research Council Research Grant, United Kingdom, 2004, 2006

Preferred Consultant, MEDACorp, Boston, Massachusetts, 2004-present

Judge, Cell Biology & Cell Signaling Section, 2nd Annual Research Symposium, Henry Ford Health System Academic Affairs, Detroit, MI, April 15, 2005

Invited Reviewer, the European Commission's Research Program on "Combating Cancer" - Apoptosis, Brussels, Belgium, 2006

Invited member of the International Advisory Committee for the International Conference on Nutrition and Cancer, Elazig, Turkey, May 20-23, 2008

Judge, Cell Biology & Cell Signaling Section, 4th Annual Research Symposium, Henry Ford Health System Academic Affairs, Detroit, MI, April 13, 2007

State and Local Boards and Committees

Department of Pharmacology, University of Pittsburgh School of Medicine

Comprehensive Examination Committee, Department of Pharmacology, University of Pittsburgh School of Medicine, 1993-1998

Committee of Graduate Studies, Department of Pharmacology, University of Pittsburgh School of Medicine, 1994-1998

Chairman of Graduate Evaluations, Department of Pharmacology, University of Pittsburgh School of Medicine, 1994-1998

NIH Predoctoral Training Grant Selection Committee, 1995

Director of Departmental Seminar Program, 1997

University of Pittsburgh School of Medicine

Competitive Medical Research Fund Review Committee (Ad Hoc Reviewer), University of Pittsburgh School of Medicine, 1996-1997

The Graduate Progress Evaluation Committee, University of Pittsburgh School of Medicine, 1997

Central Research Development Fund, University of Pittsburgh, 1997

University of South Florida and Moffitt Cancer Center & Research Institute

Member, Search Committee for Assistant Professor position in Molecular, Epidemiology Program of Cancer Control Division at Moffitt Cancer Center & Research Institute, 2001

Member, Rb Club, Moffitt Cancer Center & Research Institute, 2001-2002

Member, The Summer Intern Program Committee at Moffitt Cancer Center & Research Institute, 2002

Member, Preliminary Data Club, Moffitt Cancer Center & Research Institute, 2002-2003

Member, Proteomics Task Force Committee, Moffitt Cancer Center & Research Institute, 2002

Member, Search Committee for Structural Biology Faculty position in Drug Discovery Program at Moffitt Cancer Center & Research Institute, 2003

Member, Search Committee for the Cancer Prevention Faculty position in Molecular Epidemiology Program of Cancer Control Division at Moffitt Cancer Center & Research Institute, 2003

Member, Search Committee for Chemistry Faculty position in Drug Discovery Program at Moffitt Cancer Center & Research Institute, 2003

Member, SPARK (Summer Program for the Advancement of Research Knowledge) Selection Committee at Moffitt Cancer Center & Research Institute, 2003

Wayne State University and Karmanos Cancer Institute

Graduate student recruitment faculty for Cancer Biology Program Karmanos Cancer Institute and Wayne State University, April 3, 2004

Graduate student recruitment faculty for Cancer Biology Program Karmanos Cancer Institute and Wayne State University, April 2, 2005

Invited Reviewer, the Strategic Research Initiative Awards, Barbara Ann Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, MI, 2005

Invited Judge, the 2nd Annual Research Symposium, Henry Ford Health System, Detroit, MI, 2005

Invited Reviewer, the Seed Grants, Barbara Ann Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, MI, 2006

Co-Director and Member of a P01 Group, “AR-mediated cell cycle-dependent anti-apoptotic events in prostate cancer”, Wayne State University and Henry Ford Health System, Detroit, MI, 2004-present

Speaker (Kristin Landis from the laboratory of Q. Ping Dou), "Friends Raising Funds" program, March 5th, 2006, the Powerhouse Gym, West Bloomfield, MI.

Member, HFHS/WSU Prostate Journal Club, 2003-

Co-Director and Member of a P01 Group, “AR-mediated cell cycle-dependent anti-apoptotic events in prostate cancer”, Wayne State University and Henry Ford Health System, Detroit, MI, 2004-present

Co-Director and Member of a P01 Group focusing on nutritional agents, “Perturbation of molecular signaling by nutritional agents for cancer prevention and therapy”, Prevention Program, Karmanos Cancer Institute, Wayne State University, Detroit, MI, 2007-present

Dr. Dou has the following responsibilities:

As the leader of the Molecular Prevention sub-Program of Karmanos Cancer Institute, Dr. Dou has the following responsibilities:

- (1) Assist the Program Leader in the entire Population Studies and Prevention program and provide leadership in the Molecular Prevention sub-program, particularly with respect to basic science research, molecular targeting and chemoprevention;
- (2) Participate in Population Studies and Prevention leadership meetings;
- (3) Participate in updating and maintaining strategic planning for the Prevention sub-program;
- (4) Facilitate inter- and intra-programmatic interactions between the Prevention faculty members and members of Population Studies, Communication & Behavioral Oncology, and other cancer center programs;
- (5) Facilitate and direct nutrition/prevention-related joint grants and program projects;
- (6) Organize and lead the monthly Nutrition & Cancer Working Group meetings;
- (7) Recruit new members into the Molecular Prevention sub-program and mentor junior faculty;
- (8) Advocate for shared facilities that meet the needs of the Prevention members;
- (9) Develop the Prevention sub-program into an independent program in the next three years.

TEACHING:

Years at Wayne State University: Since August 1, 2003

Years at Other Universities:

Harvard Medical School, 1 year

University of Pittsburgh, 5 years

University of South Florida, 5 years

Courses Taught at Wayne State University

- 2003- CB 7250: CANCER CONTROL. 3 credits. 20 students
 2004 CB 7230: BREAST CANCER. 2 credits. 10-12 students (December 8, 2004, 10:00 AM- 12:00 PM, 1140 Scott Hall).
 2005- CB 7700: RECENT DEVELOPMENT IN CANCER BIOLOGY. 2 credits. ~20 students (April 11, 2005; Oct 24, 2005; Feb 12, 2007; March 25, 2008)

Courses Taught at University of South Florida

- 1999-2001 BCH 6411: Molecular Biology. Lecture. 3 credits. 25-30 students
 2001- Cancer Biology I Course Lecture. 3 credits. ~10 students

Courses Taught at University of Pittsburgh

- 1993-1998 MS MIC 2355: Advanced Molecular Genetics. Lecture and Paper Discussion. 3 credits. 8-16 students
 1993-1998 PHL 3510: Receptors and Signal Transduction. Lecture and Paper discussion. 3 credits. 10-15 students
 1993-1998 2563: Cancer Pharmacology. Lecture. 3 credits. ~5 students
 1993-1998 Medical Student Program: Problem-Based Learning Sessions. 8-10 students
 1995 Medical Student Program: Pharmacology Conference. ~20 students
 1997 Medical Student Program: Neoplasia and Neolastic Disease. 16 students
 1996-97 The Pennsylvania Governor's School Program. 6-8 students
 1997 Foundations of Biomedical Science. Small group conference. 3 credits. ~8 students

Undergraduate and Graduate/Medical Student Supervision

- 1994 Chen Yu, Harvard University, ASPET undergraduate
 1995 Peggy Lin, Penn State-Jefferson
 1995 Bill Wang, California University of PA
 1995 Vivian Lui, Department of Pharmacology, University of Pittsburgh School of Medicine, one lab rotation
 1996 Toni A. Termin, Saint Vincent College, ASPET undergraduate
 1996 Kirk E. Dineley, Department of Pharmacology, University of Pittsburgh School of Medicine, two lab rotations
1996-1999 Cheryl Fattman, Department of Pharmacology, University of Pittsburgh School of Medicine. Ph.D., Dissertation Title: "Molecular mechanisms for apoptosis-associated the retinoblastoma protein (RB) internal cleavage". Graduation with Honor (Mentor: Q. Ping Dou). Currently working as a postdoctoral fellow in Department of Pathology, University of Pittsburgh School of Medicine
 1997 Lachelle Sussman, University of New York at Buffalo, ASPET Undergraduate, University of Pittsburgh School of Medicine, one lab rotation
 1997 Kristin S Morrow, Department of Biology University of South Florida, master graduate student
 1998- Yaser S. Bassel, University of South Florida College of Medicine, medical student
 1998- Jason A. Evangelista, University of South Florida College of Medicine, medical student
 1998- Joseph J. Kavanagh, University of South Florida College of Medicine, medical student
 1998- Alexander Paloma, University of South Florida College of Medicine, medical student
 1998- Gregory A. Russell, University of South Florida College of Medicine, medical student
1999-2002 David M. Smith, Department of Biochemistry & Molecular Biology, University of South Florida College of Medicine, one lab rotation and Ph.D. student in my lab

(graduated in 2002). Dissertation Title: “Mechanistic Studies on Tumor Cell Cycle Disruption and Apoptosis by Green Tea Polyphenols and N-Thiolated beta-Lactams”. Currently working as a postdoctoral fellow in Dr. Fred Goldberg’s laboratory at Harvard Medical School)

- 1999 Lisa Smith, Department of Biology University of South Florida, undergraduate student (currently a graduate student in University of North Carolina)
- 1999 Jessica Hu, Harvard University, undergraduate student
- 1999 Daniel Lorch, University of Florida, undergraduate student
- 1999 Sun Hee Rim, Hillsborough High School, student
- 1999 Alvin Jones, Land O’Lakes High School, student
- 1999 Kristie Main, Department of Biochemistry & Molecular Biology, University of South Florida College of Medicine, one lab rotation
- 1999-2000 Kenyon Daniel, Department of Biology University of South Florida, undergraduate student. Research for Honor’s Thesis
- 2000 Lisa Smith, Moffitt Summer Intern, Department of Biology University of South Florida, undergraduate student (current graduate student at University of North Carolina)
- 2000-2002 Marie Bosley, Project LINK (Leaders In New Knowledge) Student and a Moffitt Summer Intern, Department of Biology University of South Florida, undergraduate student
- 2000 John (Chilu) Chen, Department of Biochemistry & Molecular Biology, University of South Florida College of Medicine, one lab rotation
- 2001 Jonathan S. Anderson, Moffitt Summer Intern, Zoology, University of Florida, undergraduate student
- 2001 Kyleen Charlton, Moffitt Summer Intern, Boston College, undergraduate student
- 2001-2002 Priyanka Kamath, Volunteer, Hillsborough High School, high school student
- 2000-2004 Kenyon Daniel, Department of Biochemistry & Molecular Biology, University of South Florida College of Medicine, one lab rotation and Ph.D. student in my lab (graduated in 2004). Dissertation Title: “Strategies for Cancer Therapy Through Regulation of Apoptotic Proteases”. Currently working as a postdoctoral fellow in my laboratory at Karmanos Cancer Institute, Wayne State University**
- 2001-2004 Deborah Kuhn, Cancer Biology Ph.D. Program (IOP), University of South Florida College of Medicine, one lab rotation and Ph.D. student in my lab (graduated in November of 2004). Dissertation Title: “Novel Approaches to Targeting Tumor Cell Apoptotic Signaling Pathways”. Currently working as a postdoctoral fellow in University of North Carolina.**
- 2002 Naveen Kumar, Moffitt Summer Intern, New York University, undergraduate student
- 2002 Randy Hill, Moffitt Summer Intern, University of Wisconsin, undergraduate student (currently a graduate student in University of Wisconsin)
- 2002 Seth Pross, Moffitt Summer Intern, Hillsborough High School, high school student (currently a graduate student in University of Pennsylvania)
- 2002-2003 Audrey Burns, Department of Biochemistry & Molecular Biology, University of South Florida College of Medicine, one lab rotation and Ph.D. student in my lab
- 2002-2003 Sam Falsetti, Department of Biochemistry & Molecular Biology, University of South Florida College of Medicine, one lab rotation and Ph.D. student in my lab
- 2002 Jennelle McQuown, Cancer Biology Ph.D. Program (IOP), University of South Florida College of Medicine, one lab rotation
- 2002-2003 Daniel Urbizu, Project LINK (Leaders In New Knowledge) Student, Department of Biology University of South Florida, undergraduate student

- 2003 Thomas Lendrihas, Department of Biochemistry & Molecular Biology, University of South Florida College of Medicine, one lab rotation
- 2003 Seth Pross, Moffitt Summer Intern, University of Pennsylvania, undergraduate student
- 2003 Shuoqing Song, Moffitt Summer Intern, C. Leon King High School, high school student (currently an undergraduate of MIT)
- 2003 R. Hope Harbach, Summer Student, Department of Chemistry, Eckerd College, undergraduate student
- 2003 Daniel Urbizu, Summer/Project LINK (Leaders In New Knowledge) Student, Department of Biology University of South Florida, undergraduate student
- 2003 Marina Si Chen, Summer Research Volunteer, King High School, high school student
- 2003 Sydnor M. Richkind, Summer Research Volunteer, Hillsborough High School, high school student (currently a graduate student in University of Florida)
- 2004-present Kristin Landis, Cancer Biology Program, Wayne State University, one lab rotation and Ph.D. student in my lab (defend on August 23, 2007)**
- 2004 Marina Si Chen, Summer Research Hourly Technician, Emory University, undergraduate student
- 2005-present Vesna Minic, Cancer Biology Program, Wayne State University, one lab rotation and Ph.D. student in my lab**
- 2005- Joan McCallum, Cancer Biology Program, Wayne State University, one lab rotation in my lab
- 2006-present Mike Frezza, Cancer Biology Program, Wayne State University, one lab rotation and Ph.D. student in my lab**
- 2006 Benchamart Moolmuang, Cancer Biology Program, Wayne State University, one lab rotation in my lab
- 2006 Andy Yang, Summer Research Student, Webster Thomas High School, New York
- 2006 Marina Si Chen, Summer Research Hourly Technician, Emory University, undergraduate student
- 2006 Justin Shaya, Summer Research Student, West Bloomfield High School, MI
- 2007 Azfur Ali, Summer Research Student, Troy High School, MI
- 2007 Russell TerBeek, Job-Shadowing Student, Hillsdale College, Hillsdale, MI
- 2007- Jennifer Abrams, Cancer Biology Program, Wayne State University, one lab rotation in my lab

Theses/ Dissertation or Comprehensive Examination Committees

Ph.D. Dissertation Committees

- 1995 Kirti G. Goyal, Molecular Genetics and Biochemistry, University of Pittsburgh School of Medicine (Advisor: Dr. Leaf Huang)
- 1997 Jie-Gen Jiang, Pathology, University of Pittsburgh School of Medicine (Advisor: Dr. Reza Zarnegar), graduated in 12/97
- 1997-1998 Marni Brisson, Pharmacology, University of Pittsburgh School of Medicine (Advisor: Dr. Leaf Huang)
- 1997-1998 Donald Schwartz, Pharmacology, University of Pittsburgh School of Medicine (Advisor: Dr. John Lazo)
- 1996-1998 Robert Rice, Pharmacology, University of Pittsburgh School of Medicine (Advisor: Dr. John Lazo)
- 1996-1999 Cheryl Fattman, Pharmacology, University of Pittsburgh School of Medicine (Advisor: Dr. Q. Ping Dou)

- 1999-2002 David M. Smith, Department of Biochemistry & Molecular Biology, University of South Florida College of Medicine (graduated; Ph.D. Advisor: Dr. Q. Ping Dou)
- 2000-2004 Kenyon Daniel, Department of Biochemistry & Molecular Biology, University of South Florida College of Medicine (Graduated; Ph.D. Advisor: Dr. Q. Ping Dou)
- 2001-2005 Deborah Kuhn, Cancer Biology Ph.D. Program (IOP), University of South Florida College of Arts and Sciences (Advisor: Dr. Q. Ping Dou). Graduated in November, 2004.
- 2002-2003 Audrey Burns, Department of Biochemistry & Molecular Biology, University of South Florida College of Medicine (Advisor: Dr. Q. Ping Dou)
- 2002-2003 Sam Falsetti, Department of Biochemistry & Molecular Biology, University of South Florida College of Medicine (Advisor: Dr. Q. Ping Dou)
- 2003 Bonnie Goodwin, Department of Biochemistry & Molecular Biology, University of South Florida College of Medicine (Advisor: Dr. Duane Eichler)
- 2004-07 Kristin Landis, Cancer Biology Program, Wayne State University (Advisor: Dr. Q. Ping Dou)
- 2005 Vesna Milacic, Cancer Biology Program, Wayne State University (Advisor: Dr. Q. Ping Dou)
- 2006 Mike Frezza, Cancer Biology Program, Wayne State University (Advisor: Dr. Q. Ping Dou)

Comprehensive Examination Committees

- 1994 Xiang Gao, Pharmacology, University of Pittsburgh School of Medicine
- 1995 Jeff Phillips, Pharmacology, University of Pittsburgh School of Medicine
- 1995 Chialin Cheng, Pharmacology, University of Pittsburgh School of Medicine
- 1995 Cheryl Fattman, Pharmacology, University of Pittsburgh School of Medicine
- 1996 Marni Brisson, Pharmacology, University of Pittsburgh School of Medicine
- 2003 Deborah Kuhn, Cancer Biology Ph.D. Program, University of South Florida College of Arts and Sciences

Research Associates and Others

- 2008-present Wei Huang, Visiting Scholar
- 2007-present Lily (Yan) Xiao, Visiting Scholar
- 2007-present Cindy (Xia) Zhang, Visiting Scholar
- 2006-present Carol Maconochie, Administrative Assistant (Supervisor: Dr. Q. P. Dou)
- 2005-present Huanjie Yang, Ph.D., Research Associate
- 2004-present Cindy (Qiuzhi) Cui, Technician
- 2003-2007 Di Chen, Ph.D., Research Associate
- 2007-present Di Chen, Ph.D., Research Assistant Professor

- 2007 Young Suk Jung, Ph.D., Visiting Scholar
- 2006-2007 Zhiyong Yu, Ph.D., Post-Doctoral Fellow
- 2006-2007 Guoqing Shi, Ph.D., Visiting Scholar
- 2005-2006 Jaiwei Ren, Technician
- 2004-2006 Marcianna Norris, Administrative Assistant (Supervisor: Dr. Q. Ping Dou)
- 2006 Nivedita Tiwari, M.S., Sr. Research Assistant
- 2006 Lihua Li, M.D., Visiting Scholar
- 2005-2006 Haiyan Pang, Ph.D., Research Associate
- 2005 Alejandro Diez, M.D., Physician Intern
- 2004-2005 Shirley Adanta Orlu, Research Assistant

2005-2005 Yezhou Sun, Student Assistant
2004-2005 Kenyon Daniel, Ph.D., Post-Doctoral Fellow
2003-2004 MaryAnn Sparkman, Administrative Assistant (Supervisor: Dr. Q. Ping Dou)
2003-2004 Mohammad Bhuiyan, Ph.D., Research Associate
2000-2003 Aslamuzzaman Kazi, Ph.D., Research Associate
2002 Robin Shear, Research Volunteer
2000-2002 Sherry Zhong, Research Assistant
2001-2002 Puja Gupta, Research Volunteer
2000-2001 Hongwei Wang, Research Assistant
2000-2001 Kenyon Daniel, Research Assistant
1998-2000 Sangkil Nam, Ph.D., Research Associate
2000 Gen Wang, Ph.D., Research Associate
1999-2000 Xiaoxia Zhang, M.S., Research Assistant
1998-2000 Gui Gao, Ph.D., Research Associate
1998-2000 Benyi Li, M.D., Research Associate
1998-1999 Roland Cooper, Ph.D., Research Associate
1998 Jieliu Tang, Ph.D., Research Associate
1994-1998 Bing An, Research Associate
1996-1998 Terence F. McGuire, Ph.D., Instructor
1997-1998 Yibing Peng, M.S., Research Assistant
1995-1996 Jia-Rui Jin, Visiting Scholar
1995 Leilei Zhang, Visiting Scholar

GRANT SUPPORT:

Completed support

American Cancer Society Institutional Research Grant. Cyclins, transcription and defective growth control in cancer. Principal Investigator: Qing Ping Dou. 10/01/93-06/30/95. Total Direct Costs: \$10,000

Agreement with Beth Israel Hospital. Molecular Biology of Aging. Principal Investigator: Jeanne Y. Wei. 1994. Total Direct Costs: \$10,000

NIH R01. Molecular Biology of G1/S Regulation in Murine Cells. 07/01/93-06/30/96. Subcontract Total Direct Costs: \$82,040; Total Indirect Costs: \$38,239 (Principal Investigator: Arthur B. Pardee)

NIH Shannon Award. Functions of RB-protease(s) in apoptosis. Principal Investigator: Qing Ping Dou. 09/15/95-08/31/97 (replaced by R29 on 04/14/96). Total Direct Costs: \$80,000; Total Indirect Costs: \$20,000

UPCI Breast Cancer Pilot Grant. Induction of p53-independent apoptosis and treatment of human breast cancer. Principal Investigator: Qing Ping Dou. 03/15/96-09/30/97. Total Direct Costs: \$20,000; Total Indirect Costs: \$10,200

NIH R29. Functions of RB-protease(s) in apoptosis. Principal Investigator: Q. Ping Dou (50%). 04/15/96-02/28/01. Total Direct Costs: \$349,996; Total Indirect Costs: \$178,298

NIH R01. Growth Inhibition by IL-2 of IL2R+ oral carcinomas. Principal Investigator: Q. Ping Dou (10%). 04/01/98-03/31/01. Total Direct Costs: \$105,270; Total Indirect Costs: \$44,740 (a subcontract from University of Pittsburgh)

Department of the Army Advanced Cancer Detection Center Research Grant (Moffitt). Significance of Bax-Dependent Apoptosis in Prevention and Detection of Human Prostate and Lung Cancer. Principal Investigator: Q. Ping Dou. 10/01/00-9/30/01. Total Direct Costs: \$114,773

Administrative Supplement from Moffitt Cancer control. Co-Principal Investigator: Q. Ping Dou. \$4,285

Administrative Supplement from Moffitt Foundation. Co-Principal Investigator: Q. Ping Dou. \$2,300

Agreement from University of North Texas. Co-Principal Investigator: Q. Ping Dou. \$3,000

NIH R03. Tea Targeting Proteasome: A Role in Cancer Prevention. Principal Investigator: Q. Ping Dou (10%). 07/01/01-06/30/03. Total Direct Costs: \$100,000; Total Indirect Costs: \$45,000

Supplement for Correlative Studies Related to estrogen Receptor Negative (ER-negative) Breast Cancer (Moffitt CCOP Research Base). PI: Krischer; Co-Investigator: Q. Ping Dou. \$36,040

U10 CA81920. A Clinical Trial of the Action of Isoflavones in Breast Neoplasia: Administration Prior to Mastectomy or Lumpectomy - A Pilot Study. PI: Krischer; Co-Investigator: Q. Ping Dou. 12/01/01-11/30/03

U10 CA81920. The Specific Role of Isoflavones in reducing Prostate Cancer Risks. PI: Krischer; Co-Investigator: Q. Ping Dou. 12/01/01-11/30/03

U10 CA81920. A Randomized Pilot Clinical Trial of the Action of Isoflavones and Lycopene in Localized Prostate Cancer: Administration Prior to radical Prostatectomy. PI: Krischer; Co-Investigator: Q. Ping Dou. 12/01/01-11/30/03

Approved but not funded

American Cancer Society. Induction of an RB-associated phosphatase and cancer cell apoptosis (**Score: the second decile**). Principal Investigator: Q. Ping Dou. 01/01/97-12/31/99. Total Direct Costs: \$252,151; Total Indirect Costs: \$63,038

American Institute for Cancer Research. Tea polyphenols target proteasome-mediated Bax degradation pathway: Significance in prostate cancer prevention and treatment (**Score: 2.92**). Principal Investigator: Q. Ping Dou. 02/01/00-01/31/03. Total Direct Costs: \$150,000; Total Indirect Costs: \$15,000

Present support

NIH R01. Roles of polymorphic COMT, tea polyphenols and proteasome in cancer prevention. 20% Effort (PI: Q. Ping Dou). 04/01/06-03/31/11. Total Direct Costs: \$912,550; Total Indirect Costs: \$460,842

DOD Breast Cancer Research Program/IDEA Award. Synthetic β -Lactam Antibiotics as A Selective Breast Cancer Cell Apoptosis Inducer: Significance in Breast Cancer Prevention and Treatment. 20% Effort (PI: Q. Ping Dou). 03/01/04-3/31/08. Total Direct Costs: \$350,626; Total Indirect Costs: \$115,464

NIH R01. N-Thiolated β -Lactams. Co-Principal Investigator: Q. Ping Dou (5%) (PI: Ed Turos). 03/01/02-02/28/07. Total Direct Costs (to Dou lab): \$200,000; Total Indirect Costs: \$90,000

DOD Breast Cancer Research Program-Concept Award. Examination of potential anti-tumor activity of N-thiolated β -lactam antibiotics in nude mice bearing human breast tumors. 5% Effort (PI: Q. Ping Dou). 10/01/04-09/30/06. Total Direct Costs: \$75,000; Total Indirect Costs: \$38,250

NIH R03. The Proteasome as Molecular Target of Grape Polyphenols. 5% Effort (PI: Q. Ping Dou). 12/01/04-11/30/06. Total Direct Costs: \$100,000; Total Indirect Costs: \$51,000

Wayne State University President's Research Enhancement Program Proposal. Enhancing chemo- and photodynamic therapy in breast cancer using nanotechnology. (Co-I: Q. Ping Dou; PI: Jayanth Panyam). 06/01/06-05/31/08. Total Direct Costs (Dou Lab): \$64,856

VA Merit. Ubiquitin Protease System in Malignant Pleural Mesothelioma. 10% Effort. (Co-I: Q. Ping Dou; PI: Anil Wali). 09/01/06-08/31/10. Total Direct Costs: \$795,600

NIH R21. Dual-agent nanoparticles to overcome drug resistance. 3% Effort (Co-PI: Q. Ping Dou; PI: Jayanth Panyam). 07/01/07-06/30/09. Total Direct Costs: \$250,000; Total Indirect Costs: \$126,250 (**2.8 percentile**)

NIEHS P50 ES012395. Center for Urban African American Health. 3.0% Effort (Co-I: Q. Ping Dou; PI: John Flack). 06/01/05-05/31/07.

T32-CA09531-19 NIH Training Grant. "Training Program in the Biology of Cancer" (Ms. Kristin R. Landis-Piwowar; Mentor: Q. Ping Dou) 09/01/05-8/31/07.

NCI/NIH the Cancer Center Support Grant (PI: Ruckdeschel). Population Studies & Prevention Program (Program PI: Schwartz; Co-I: Q. Ping Dou, 5%) 10/01/05-9/31/10.

T32-CA09531 NIH Training Grant. "Training Program in the Biology of Cancer" (Mr. Michael Frezza; Mentor: Q. Ping Dou) 09/01/07-8/31/09.

Karmanos Cancer Institute Startup funds. Principal Investigator: Q. Ping Dou. 08/01/03 -.

Karmanos Cancer Institute Indirect Account. Principal Investigator: Q. Ping Dou. 08/01/03 -.

Pending support

NIH R01. Elucidation of molecular mechanisms for the ubiquitin-proteasome pathway in malignant pleural mesothelioma (Co-I: Q. Ping Dou; PI: Anil Wali). 10/01/05-9/30/10. Total Direct Costs: \$1,250,000; Total Indirect Costs: \$586,500

NIH R21. Molecular Study on Novel NCI Potential Anti-tumor Drugs. 15% Effort (PI: Q. Ping Dou). 04/01/05-03/31/07. Total Direct Costs: \$200,000; Total Indirect Costs: \$102,000

NIH R03. Targeting tumor endogenous copper with clioquinol. 5% Effort (PI: Q. Ping Dou). 07/01/05-06/30/07. Total Direct Costs: \$100,000; Total Indirect Costs: \$51,000

NIH R21. Dual-agent nanoparticles to overcome drug resistance. 5% Effort (Co-PI: Q. Ping Dou; PI: Jayanth Panyam). 07/01/05-06/30/07. Total Direct Costs: \$250,000; Total Indirect Costs: \$124,950

Alliance For Cancer Gene Therapy. Dual-agent nanoparticles to overcome drug resistance. 5% Effort (Co-PI: Q. Ping Dou; PI: Jayanth Panyam). 07/01/05-06/30/08. Total Direct Costs: \$449,991; Total Indirect Costs: \$44,999

DOD Breast Cancer Research Program-Concept Award. Determination of potential anti-cancer activity of synthetic acetylated EGCG analog prodrugs in nude mice bearing human breast tumor xenografts. 5% Effort (PI: Q. Ping Dou). 07/01/05-06/30/06. Total Direct Costs: \$75,000; Total Indirect Costs: \$38,250

DOD Breast Cancer Research Program-Concept Award. Synchronized Gene Silencing and Drug Delivery to Overcome Drug Resistance in Breast Cancer. 5% Effort (Co-PI: Q. Ping Dou; PI: Jayanth Panyam). 07/01/05-06/30/06. Total Direct Costs: \$75,000; Total Indirect Costs: \$38,250

NIH R01. Synthesis and evaluation of prodrugs of green tea polyphenol EGCG analogs. 20% Effort (PI: Q. Ping Dou). 12/01/05-11/30/10. Total Direct Costs: \$1,250,000; Total Indirect Costs: \$380,460

DOD Prostate Cancer Research Program-Idea Development Award. MOLECULAR TARGETS OF NOVEL NCI POTENTIAL ANTICANCER DRUGS IN HUMAN PROSTATE CANCER CELLS. 20% Effort (PI: Q. Ping Dou). 10/01/05-09/30/08. Total Direct Costs: \$375,000; Total Indirect Costs: \$191,250

The Michigan Technology Tri-Corridor Fund, Fiscal Year 2005 Competition. DUAL-AGENT NANOPARTICLES TO OVERCOME DRUG RESISTANCE. 5% Effort (Co-PI: Q. Ping Dou; PI: Jayanth Panyam). 07/01/05-06/30/07. Total Direct Costs: \$85,320 (to Dou Lab)

DOD Breast Cancer Research Program/IDEA Award. The potential use of the anti-alcoholism drug disulfiram in breast cancer prevention and treatment. 15% Effort (PI: Q. Ping Dou). 01/01/06-12/31/08. Total Direct Costs: \$300,000; Total Indirect Costs: \$146,403

DOD Prostate Cancer Research Program/Physician Research Training Award. Novel organic copper complex PDC-Cu for molecular therapy of prostate cancer facilitated by PET imaging (PI: Fangyu Peng; Mentor: Q. Ping Dou). 10/01/05-09/30/10. Total Direct Costs: \$634,445; Total Indirect Costs: \$50,756

Susan Komen Foundation. Nanoparticle-mediated combination photodynamic and chemotherapy to overcome refractory tumors. 5% Effort (Co-PI: Q. Ping Dou; PI: Jayanth Panyam). 05/01/06-04/30/08. Total Direct Costs: \$200,000

Wilson Foundation. Targeting tumor endogenous copper with the antibiotic clioquinol: A novel approach for cancer-specific killing with no or low toxicity. 15% Effort (PI: Q. Ping Dou). 01/01/06-12/31/07. Total Direct Costs: \$200,000

VA Merit. Ubiquitin Protease System in Malignant Pleural Mesothelioma. 10% Effort. (Co-I: Q. Ping Dou; PI: Anil Wali). Total Direct Costs: \$795,600

NIH R01. Copper as a novel target for determining fate of AR and prostate cancer cells. 20% Effort (PI: Q. Ping Dou). 07/01/06-06/30/11. Total Direct Costs: \$1,250,000; Total Indirect Costs: \$628,693

NIH R21 (resubmission). Dual-agent nanoparticles to overcome drug resistance. 5% Effort (Co-PI: Q. Ping Dou; PI: Jayanth Panyam). 07/01/06-06/30/08. Total Direct Costs: \$250,000; Total Indirect Costs: \$126,250

NIH R01. The Chinese Thunder of God Vine: Active Components & Biological Targets in Cancer. 20% Effort (PI: Q. Ping Dou). 12/01/06-11/30/11. Total Direct Costs: \$1,250,000; Total Indirect Costs: \$568,125

NIH P01. AR-mediated cell cycle-dependent anti-apoptotic events in prostate cancer. (PI: Reddy GPV; Co-Directors: Dou QP and Menon M). Project #3: Targeting 26S proteasome for determining fate of AR and prostate cancer cells. 40% Effort (PI: Q. Ping Dou). 12/01/06-11/30/11. Total Direct Costs: \$4,516,015 (total for KCI/WSU); Total Indirect Costs: \$2,247,665 (total for KCI/WSU).

DOD Breast Cancer Research Program-Concept Award. Chemosensitization of human breast cancer cells by an active compound purified from the Chinese medicine Thunder of God vine. 5% Effort (PI: Q. Ping Dou). 07/01/06-06/30/07. Total Direct Costs: \$75,000; Total Indirect Costs: \$37,875

NIH R01. Maspin in Hormone Refractory Prostate Cancer Intervention (Co-I: Q. Ping Dou, 5%; PI: Shijie Sheng). 12/01/06-11/30/11. Total Direct Costs: \$1,250,000; Total Indirect Costs: \$568,125

NIH R01 (resubmission). Elucidation of molecular mechanisms for the ubiquitin-proteasome pathway in malignant pleural mesothelioma (Co-I: Q. Ping Dou; PI: Anil Wali). 12/01/06-11/30/11. Total Direct Costs: \$1,250,000; Total Indirect Costs: \$586,500

MICHIGAN ECONOMIC DEVELOPMENT CORPORATION (MEDC). Development of natural pharmaceuticals to protect against low-intensity radiation exposure. 5% Effort (Co-PI: Q. Ping Dou; PI: Michael C Joiner). 10/01/06-09/30/09. Total Direct Costs: \$1,026,000; Total Indirect Costs: \$153,900

National Natural Science Foundation of China (NSFC). Synthesis and Mechanistic Study of Catechin Glycosides as Proteasome Inhibitors. Co-PI: Q. Ping Dou (PI: Sheng Biao Wan). 10/01/06-09/30/08.

DOD Prostate Cancer Research Program-Idea Development Award. Targeting the proteasome/ NF κ B/ Androgen receptor-mediated survival pathway to chemosensitize human prostate cancer cells. 20% Effort (PI: Q. Ping Dou). 12/01/06-11/30/09. Total Direct Costs: \$375,000; Total Indirect Costs: \$189,375.

DOD Breast Cancer Research Program/IDEA Award. Overcoming breast cancer drug resistance by a medicinal compound isolated from Indian Winter Cherry. 15% Effort (PI: Q. Ping Dou). 12/01/06-11/30/09. Total Direct Costs: \$300,000; Total Indirect Costs: \$151,500

Wayne State University Faculty Graduate Research Assistantship (GRA). Gold-Containing Compounds as Proteasome Inhibitors and Apoptosis Inducers and their Potential Use for Breast Cancer Treatment. 08/17/07-08/16/08. Total Direct Costs: \$18,000.

Fund for Cancer Research. Targeting the proteasome/ NF κ B/ Androgen receptor-mediated survival pathway to chemosensitize human prostate cancer cells. (PI: Q. Ping Dou). 02/01/07-01/31/08. Total Direct Costs: \$75,000.

NIH R01. Chemoprevention of Malignant Mesothelioma (Co-I: Q. Ping Dou, 5%; PI: Anil Wali). 10/01/07-9/30/12. Total Direct Costs: \$1,250,000; Total Indirect Costs: \$586,500

The Harry A. and Margaret D. Towsley Foundation. Prevention Program Research Projects. (PI: Q. Ping Dou). 01/01/08-12/31/12. Total Direct Costs: \$1,050,000.

National Natural Science Foundation of China (NSFC). Synthesis and structure-activity-relationship study of proteasome-targeted catechin analogs and their antitumor activities. Co-PI: Q. Ping Dou (PI: Sheng Biao Wan). 10/01/07-09/30/09.

EHS Center in Molecular and Cellular Toxicology with Human Applications at Wayne State University. Targeting the carcinogenic pollutant cadmium in human breast cancer cells. (PI: Q. Ping Dou). 05/01/07-04/30/08. Total Direct Costs: \$35,000.

National Natural Science Foundation of China (NSFC). Research for New Anti-Cancer Based on Small Molecular Biomarker of Celastrol Analogue Targeted on Proteasome in Cancer. Co-PI: Q. Ping Dou (PI: Xiao Yuan). 10/01/07-09/30/09.

Karmanos Cancer Institute Strategic Research Initiative Grant. Chemosensitization of human prostate cancer cells by simultaneous inhibition of two distinct survival pathways, the proteasome and EGFRs. 10% Effort (PI: Q. Ping Dou; Co-PI: Adhip Majumdar). 05/01/07-04/30/08. Total Direct Costs: \$100,000

Karmanos Cancer Institute Strategic Research Initiative Grant. Oncogenic Properties and therapeutic potential of *PIK3CA* mutations in Breast Cancer. 10% Effort (Co-PI: Q. Ping Dou; PI: Guojun Wu). 05/01/07-04/30/08. Total Direct Costs: \$100,000

DOD Breast Cancer Research Program/IDEA Award. Investigation of oncogenic properties and therapeutic application of *PIK3CA* mutations in breast cancer. 5% Effort (Co-I: Q. Ping Dou; PI: Guojun Wu). 12/01/07-11/30/10. Total Direct Costs: \$300,000; Total Indirect Costs: \$151,500

Susan Kumen Foundation. Investigation of oncogenic properties and therapeutic application of *PIK3CA* mutations in breast cancer. 5% Effort (Co-I: Q. Ping Dou; PI: Guojun Wu). 12/01/07-11/30/10. Total Direct Costs: \$360,000; Total Indirect Costs: \$90,000

Susan Kumen Foundation. Overcome human breast cancer drug resistance by Disulfiram, a clinically used anti-alcoholism drug. 10% Effort (Co-PI: Q. Ping Dou; PI: Di Chen). 12/01/07-11/30/10. Total Direct Costs: \$360,000; Total Indirect Costs: \$90,000

EHS Center in Molecular and Cellular Toxicology with Human Applications at Wayne State University. Targeting the carcinogenic pollutant organotins. (PI: Q. Ping Dou). 03/01/08-02/29/09. Total Direct Costs: \$25,000.

DOD Breast Cancer Research Program-Concept Award. Combinational treatment of breast cancer with increased potency and selectivity. 10% Effort (PI: Q. Ping Dou). 07/01/08-06/30/09. Total Direct Costs: \$75,000; Total Indirect Costs: \$37,875

DOD Breast Cancer Research Program-Concept Award. Reverse drug resistance in human breast cancer cells by Disulfiram. 5% Effort (Co-PI: Q. Ping Dou; PI: Di Chen). 07/01/08-06/30/09. Total Direct Costs: \$75,000; Total Indirect Costs: \$37,875

DOD Prostate Cancer Research Program-Idea Development Award. TBN. 20% Effort (PI: Q. Ping Dou). 10/01/08-09/30/11. Total Direct Costs: \$375,000; Total Indirect Costs: \$191,250

Multiple Myeloma Research Foundation. EGCG and Myeloma. 5% Effort (Co-PI: Q. Ping Dou; PI: Jeff Zonder). 05/01/08-04/30/10. Total Direct Costs: \$90,000; Total Indirect Costs: \$45,000

National Natural Science Foundation of China (NSFC). Synthesis and structure-activity-relationship study of proteasome-targeted catechin analogs and their antitumor activities. Co-PI: Q. Ping Dou (PI: Sheng Biao Wan). 10/01/08-09/30/10.

DOD Breast Cancer Research Program/IDEA Award. The potential use of an anti-AIDS drug in breast cancer prevention and treatment. 15% Effort (PI: Q. Ping Dou). 09/01/08-08/31/11. Total Direct Costs: \$375,000; Total Indirect Costs: \$18,938.

DOD Prostate Cancer Research Program-Idea Development Award. Synthesis and Biological Evaluation of DSF analogs for treatment of prostate cancer cells. 20% Effort (PI: Q. Ping Dou). 12/01/08-11/30/11. Total Direct Costs: \$375,000; Total Indirect Costs: \$189,378

The Office of International Programs at Wayne State University. Breast cancer research project related to international research and/or faculty exchanges. PI: Q. Ping Dou. 05/01/08-09/30/08. Total Direct Costs: \$6,000

PUBLICATIONS:

Original Observations in Referred Journals

1. Chen KY and Dou QP. NAD⁺ stimulated the spermidine-dependent hypusine formation on the 18,000-dalton protein in cytosolic lysates derived from NB-15 mouse neuroblastoma cells. FEBS Letters, 1988; 229: 325-328
2. Chen KY and Dou QP. Isolation and characterization of an 18,000-dalton hypusine-containing protein from NB-15 mouse neuroblastoma cells. Biochem. Biophys. Acts, 1988; 971: 21-28
3. Dou QP and Chen KY. Two hypusine-containing proteins identified by metabolic labeling in chick embryo fibroblasts. J. Chin. Chem. Soc., 1989; 36: 443-450

4. Dou QP and Chen KY. Characterization and reconstitution of a cell-free system for NAD⁺-dependent deoxyhypusine formation on the 18 kDa eIF-4D precursor. *Biochem. Biophys. Acts*, 1990; 1036: 128-137
5. Bradley DW, Dou QP, Fridovich-Keil JL and Pardee AB. Transformed and non-transformed cells differ in stability and cell cycle regulation of a binding activity to the thymidine kinase promoter. *Proc. Natl. Acad. Sci. USA*, 1990; 87: 9310-9314
6. Dou QP, Fridovich-Keil JL and Pardee AB. Inducible proteins binding to the murine thymidine kinase promoter in late G1/S phase. *Proc. Natl. Acad. Sci. USA*, 1991; 88: 1157-1161
7. Fridovich-Keil JL, Gudas JM, Dou QP, Bouvard I and Pardee AB. Growth-responsive expression from the murine thymidine kinase promoter: Genetic analysis of DNA sequences. *Cell Growth. Diff.*, 1991; 2: 67-76
8. Dou QP, Markell PJ, and Pardee AB. Thymidine kinase transcription is regulated at G1/S phase by a complex that contains retinoblastoma-like protein and cdc2 kinase. *Proc. Natl. Acad. Sci. USA*, 1992; 89: 3256-3260
9. Dou QP, Levin AH, Zhao S, and Pardee AB. Cyclin E and cyclin A as candidates for the restriction point protein. *Cancer Res. (Advances in Brief)*, 1993; 53: 1493-1497
10. Dou QP, Zhao S, Levin AH, Wang J, Helin K, and Pardee AB. G1/S-regulated E2F-containing complexes bind to the mouse thymidine kinase gene promoter. *J. Biol. Chem.*, 1994; 269: 1306-1313
11. Dou QP, Molnar G, and Pardee AB. Cyclin D1/cdk2 kinase is present in a G1 phase-specific protein complex Y1 that binds to the mouse thymidine kinase gene promoter. *Biochem. Biophys. Res. Commun.*, 1994; 205: 1859-1868
12. Dou QP, An B and Will PL. Induction of a retinoblastoma phosphatase activity by anticancer drugs accompanies p53-independent G1 arrest and apoptosis. *Proc. Natl. Acad. Sci. USA*, 1995; 92: 9019-9023
13. Dou QP, An B and Yu C. Activation of cyclin E-dependent kinase by DNA-damaging signals during apoptosis. *Biochem. Biophys. Res. Commun.*, 1995; 214: 771-780
14. Dou QP and Lui VWY. Failure to dephosphorylate retinoblastoma protein in drug resistant cells. *Cancer Res. (Advances in Brief)*, 1995; 55: 5222-5225
15. An B and Dou QP. Cleavage of retinoblastoma protein during apoptosis: an interleukin 1 β -converting enzyme-like protease as candidate. *Cancer Res. (Advances in Brief)*, 1996; 56: 438-442
16. Dou QP, Pardee AB and Keyomarsi K. Cyclin E—a better prognostic marker for breast cancer than cyclin D? *Nature Medicine*, 1996; 2: 254
17. An B, Jin J-R, Lin P and Dou QP. Failure to activate interleukin 1 β -converting enzyme-like proteases and to cleave retinoblastoma protein in drug-resistant cells. *FEBS Letters*; 1996; 399: 158-162
18. Dou QP, An B, Antoku K and Johnson DE. Fas stimulation induces RB dephosphorylation and proteolysis that is blocked by inhibitors of the ICE protease family. *J. Cell. Biochem.*, 1997; 64: 586-594
19. Molnar GM, Crozat A, Kraeft S-K, Dou QP, Chen LB and Pardee AB. Association of the mammalian helicase MAH with the pre-mRNA splicing complex. *Proc. Natl. Acad. Sci. USA*, 1997; 94: 7831-7836
20. Fattman CL, An B and Dou QP. Characterization of interior cleavage of retinoblastoma protein in apoptosis. *J. Cell. Biochem.* (A figure was selected as the cover of the journal), 1997; 67: 399-408
21. An B, Dineley KE, Zhang LL, Termin TA, Meijer L and Dou QP. Involvement of RB kinases and phosphatases in life and death decisions. *Oncology Reports*, 1997; 4: 1129-1134

22. An B, Johnson DE, Jin J-R, Antoku K and Dou QP. Bcl-2- and CrmA-inhibitable dephosphorylation and cleavage of retinoblastoma protein during etoposide-induced apoptosis. *Intl. J. Mol. Med.*, 1998; 1: 131-136
23. Fattman CL, An B, Sussman L and Dou QP. p53-independent dephosphorylation and cleavage of retinoblastoma protein during tamoxifen-induced apoptosis in human breast carcinoma cells. *Cancer Lett.*, 1998; 130: 103-113
24. An B, Goldfarb RH, Siman R and Dou QP. Novel dipeptidyl proteasome inhibitors overcome Bcl-2 protective function and selectively accumulate the cyclin-dependent kinase inhibitor p27 and induce apoptosis in transformed, but not normal, human fibroblasts. *Cell Death & Diff.*, 1998; 5: 1062-1075
25. Dou QP, McGuire TF, Peng Y and An B. Proteasome inhibition leads to significant reduction of Bcr-Abl expression and subsequent induction of apoptosis in K562 human chronic myelogenous leukemia cells. *J. Pharm. Exp. Ther.*, 1999; 289: 781-790
26. Reichert TE, Nagashima S, Kashii Y, Stanson J, Gao G, Dou QP and Whiteside TL. Interleukin-2 expression in human carcinoma cell lines and its role in cell cycle progression. *Oncogene*, 2000; 19: 514-525
27. Li B and Dou QP. Bax degradation by the ubiquitin/proteasome-dependent pathway: involvement in tumor survival and progression. *Proc. Natl. Acad. Sci. USA*, 2000; 97: 3850-3855
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- Smith DM, Nam S and Dou QP. Studies on tumor related targets of green tea polyphenols. Poster presentation. 2nd International Conference. Mechanisms of Cell Death and Disease: Advances in Therapeutic Intervention, North Falmouth, MA, June 2-6, 2001
- Daniel KG, Zhong Q, Gupta P and Dou QP. Etoposide induces activation of calpain in early stages of apoptosis. Poster presentation. 22nd Annual South East Pharmacology Society Meeting (SEPS), Drug Development Symposium (from Bench to Bedside), Clearwater, FL, October 4-6, 2001
- Kazi A, Smith DM, Zhong Q and Dou QP. Down Regulation of Bcl-X_L Protein Expression by Green Tea Polyphenols Is Associated with Prostate Cancer Cell Apoptosis. Poster presentation. AACR-NCI-EORTC. Molecular Targets and Cancer therapeutics, Miami Beach, FL, October 29-November 2, 2001
- Dou QP, Kazi A, Smith DM and Kuhn DJ. Tea Polyphenols Target Proteasome-Mediated Bax Degradation in Prostate Cancer Cells: A Potential Role in Cancer Prevention. Poster presentation. An AACR Special Conference in Cancer Research. New Discoveries in Prostate Cancer Biology and Treatment, Naples, FL, December 5-9, 2001

- Nam S, Dalton WS, Trotti AM, Dou QP and Calvin DP. Cell adhesion to fibronectin (FN) through $\beta 1$ integrins results in cell adhesion mediated ionizing radiation resistance (CAM-RR) in human LNCaP prostate cancer cells: the potential involvement of proteasome chymotrypsin-like activity. Poster presentation. AACR 93rd Annual Meeting, San Francisco, CA, April 6-10, 2002
- Kazi A, Hill R, Long TE, Turos E, Dou QP. Selective induction of apoptosis in human tumor cells by novel N-thiolated beta-lactams. Poster presentation. Molecular Targets for Cancer Therapy, St. Pete Beach, FL, October 11-15, 2002
- Nam S, Dalton WS, Dou QP, Jove R and Calvin DP. Proteasome chymotrypsin-like activity (PCA) is implicated in LNCaP prostate cancer cell adhesion mediated ionizing radiation (IR) resistance (CAM-RR). Poster presentation. Molecular Targets for Cancer Therapy, St. Pete Beach, FL, October 11-15, 2002
- David M. Smith, Zhigang Wang, Aslamuzzaman Kazi, Kenyon G. Daniel, Lian-hai Li, Tak-Hang Chan, and Q. Ping Dou. Green tea polyphenol proteasome inhibitors as potential cancer-preventative agents: computational design, organic synthesis and biological evaluation. Poster presentation. AACR Special Conference, Proteases, Extracellular Matrix, and Cancer, Hilton Head Island, South Carolina, October 9-13, 2002
- Lu M, Dou QP, Kitson RP, Smith DM, and Goldfarb RH. Differential Effects of Proteasome Inhibitors on Cell Cycle Progression and Molecular Modulation in Human Natural Killer Cells and T Lymphocytes. AAI, 2003
- Kazi A, Hill R, Long TE, Turos E, and Dou QP. Selective Induction of Apoptosis in Human Tumor Cells by Novel N-thiolated Beta-Lactams. Poster presentation. Moffitt Scientific Retreat, Tampa, FL, February 15, 2003
- Smith DM, Wang Z, Kazi A, Daniel KG Li LH, Chan TH and Dou QP. Green tea polyphenol proteasome inhibitors as potential cancer-preventative agents: computational design, organic, synthesis and biological evaluation. Poster presentation. Moffitt Scientific Retreat, Tampa, FL, February 15, 2003
- Minic V, Kuhn D, Daniel K, Chen D, Landis K, Turos E, Dou QP. Potential Use of β -Lactam antibiotics in Cancer Prevention and Therapy. Poster presentation. Cancer Biology Program, Karmanos Cancer Institute and Wayne State University, Detroit, MI, September 8, 2004
- Di Chen, Marina Chen, Kenyon G. Daniel, Deborah J. Kuhn, Kristin Landis, and Q. Ping Dou. Dietary Flavonoids as Proteasome Inhibitors and Apoptosis Inducers in Human Leukemia Cells and Their Structure-activity Relationships. Poster presentation. Third Annual AACR Cancer Prevention Meeting, Seattle, Washington, October 16-20, 2004
- Deborah Kuhn, Yang Wang, Vesna Minic, Cristina Coates, G. Suresh Kumar Reddy, Kenyon, G. Daniel, Jeung-Yeop Shim, Di Chen, Kristin R. Landis-Piwowar, Edward Turos, And Q. Ping Dou, Ph.D. Relationships of structures of N-methylthiolated beta-lactam antibiotics to their apoptosis-inducing activity in human breast cancer cells. Poster presentation. ERA OF HOPE, Department of Defense (DOD) Breast Cancer Research Program (BCRP) Meeting, Pennsylvania Convention Center, Philadelphia, Pennsylvania, June 8-11, 2005
- Minic V, Kuhn D, Daniel K, Chen D, Landis K, Turos E, Dou QP. Potential Use of β -Lactam antibiotics in Cancer Prevention and Therapy. Poster presentation. Graduate student recruitment for Cancer Biology Program, Karmanos Cancer Institute and Wayne State University, April 2, 2005
- Di Chen, Marina Chen, Kenyon G. Daniel, Deborah J. Kuhn, Kristin Landis, and Q. Ping Dou. Dietary Flavonoids as Proteasome Inhibitors and Apoptosis Inducers in Human Leukemia Cells and Their Structure-activity Relationships. Poster presentation. Graduate student recruitment for Cancer Biology Program, Karmanos Cancer Institute and Wayne State University, April 2, 2005
- Kristin R. Landis-Piwowar, Deborah J. Kuhn, Sheng Biao Wan, Di Chen, Tak Hang Chan, and Q. Ping Dou. Evaluation of Proteasome-Inhibitory and Apoptosis-inducing Potencies of Novel (-)-

- EGCG Analogs and their Prodrugs. Poster presentation. Graduate student recruitment for Cancer Biology Program, Karmanos Cancer Institute and Wayne State University, April 2, 2005
- Di Chen, Kenyon G. Daniel, Marina S. Chen, Deborah J. Kuhn, Kristin R. Landis Piwowar, Wai Har Lam, Larry M. C. Chow, Tak Hang Chan and Q. Ping Dou. Dietary and synthetic polyphenols as proteasome inhibitors and apoptosis inducers in human cancer cells. 5th Annual Symposium, Michigan Prostate Research Colloquium, Basic and Clinical Advances in Prostate Cancer Research, Wayne State University School of Medicine, Detroit, MI 48201, April 23, 2005
- Di Chen, Qiuzhi Cindy Cui, Huanjie Yang, Fazlul H. Sarkar, G. Prem Veer Reddy, Shijie Sheng, Raul A Barrea and Q. Ping Dou. Clioquinol, A Therapeutic Agent For Alzheimer's Disease, Has Proteasome-Inhibitory, Apoptosis-Inducing And Anti-Tumor Activities In Prostate Cancer Cells And Xenografts. 6th Annual Symposium, Michigan Prostate Research Colloquium, Basic and Clinical Advances in Prostate Cancer Research, University of Michigan Medical School, Towsley Center, Dow Auditorium, Ann Arbor, MI 48109, May 6, 2006
- Huanjie Yang, Di Chen, Qiuzhi Cindy Cui, Xiao Yuan, and Q. Ping Dou. Celastrol, A Triterpene Extracted From The Chinese Thunder Of God Vine, Is A Potent Proteasome Inhibitor And Suppresses Human Prostate Cancer Growth In Nude Mice. 6th Annual Symposium, Michigan Prostate Research Colloquium, Basic and Clinical Advances in Prostate Cancer Research, University of Michigan Medical School, Towsley Center, Dow Auditorium, Ann Arbor, MI 48109, May 6, 2006 (The First Place Poster Award)**
- Xiaohua Li, Di Chen, Shuping Yin, Yiwei Li, Huanjie Yang, Kristin R. Landis-Piwowar, Fazlul Sarkar, Prem Veer G. Reddy, Q. Ping Dou, Shijie Sheng. Proteasome Inhibition Up-regulates Apoptosis-sensitizing Maspin. 6th Annual Symposium, Michigan Prostate Research Colloquium, Basic and Clinical Advances in Prostate Cancer Research, University of Michigan Medical School, Towsley Center, Dow Auditorium, Ann Arbor, MI 48109, May 6, 2006
- Di Chen, Qiuzhi Cindy Cui, Huanjie Yang and Q. Ping Dou. Disulfiram, a clinically used anti-alcoholism drug and copper-binding agent, induces apoptotic cell death in breast cancer cultures and xenografts *via* inhibition of the proteasome activity. The AACR International Conference on Frontiers in Cancer Prevention Research, Boston, MA, November 12 - 15, 2006.
- Kristin R. Landis-Piwowar, Deborah J. Kuhn, Sheng Biao Wan, Di Chen, Tak Hang Chan, and Q. Ping Dou. Evaluation of Proteasome-Inhibitory and Apoptosis-inducing Potencies of Novel (-)-EGCG Analogs and their Prodrugs. Poster presentation. Karmanos Cancer Institute the Protease and Cancer Program Annual Retreat, Scott Hall, Wayne State University, Detroit, MI, December 1, 2006
- Landis-Piwowar KR, Chen D, Chen M, Cui QZ, Dou QP. The dietary flavonoid apigenin inhibits proteasome activity and suppresses breast cancer growth in nude mice. Poster Presentation. In the Forefront of Basic and Translational Cancer Research, AACR/JCA 7th Joint Conference, Waikoloa, HI, January 21 - 25, 2007.
- Khdair A, Chen D, Dou QP, Panyam J. Nanoparticle-mediated Chemo- and Photodynamic Therapy for Drug Resistant Tumors. Poster Presentation. 10th Annual Meeting of Regional Cancer Center Consortium for the Biological Therapy of Cancer, International Hotel and MBNA Conference Center, Cleveland, Ohio, February 8-9, 2007.
- Di Chen, Qiuzhi Cindy Cui, Huanjie Yang, Raul A Barrea, Fazlul H. Sarkar, Shijie Sheng, Bing Yan, G. Prem Veer Reddy, and Q. Ping Dou. Clioquinol, a therapeutic agent for Alzheimer's disease, has proteasome-inhibitory, androgen receptor-suppressing, apoptosis-inducing, anti-angiogenic and anti-tumor activities in human prostate cancer cells and xenografts. The 2007 AACR Annual Meeting, Los Angeles, CA, April 14 - 18, 2007.
- Raul A Barrea, Di Chen, Qiuzhi Cindy Cui, Huanjie Yang, Fazlul H. Sarkar, Shijie Sheng, Bing Yan, G. Prem Veer Reddy, and Q. Ping Dou. Copper accumulation in Cancer, but not Normal

- Tissues reveals potential tumor-specific killing of Cu ligand Clioquinol. The 2007 Users Week at Argonne National Laboratory, Argonne, IL, May 7-12, 2007
- Yang HJ, Landis-Piwowar KR, Lu DY, Yuan P, Li LH, Reddy GPV, Yuan Xiao, and Dou QP. Pristimerin Induces Apoptosis by Targeting the Proteasome in Prostate Cancer Cells. 7th Annual Symposium, Michigan Prostate Research Colloquium, Basic and Clinical Advances in Prostate Cancer Research, Henry Ford Health System, One Ford Place, Detroit, MI, May 12, 2007.
- Chen D, Cui QC, Yang HJ, Barrea RA, Sarkar FH, Sheng S, Yan B, Reddy GPV and Dou QP. Clioquinol, a Therapeutic Agent for Alzheimer's Disease, Has Proteasome-inhibitory, Androgen Receptor Suppressing, Apoptosis-inducing and Anti-tumor Activities in Human Prostate Cancer Cells and Xenograft. 7th Annual Symposium, Michigan Prostate Research Colloquium, Basic and Clinical Advances in Prostate Cancer Research, Henry Ford Health System, One Ford Place, Detroit, MI, May 12, 2007.
- Chen D, Frezza M, Shakya R, Cui QZ, Milacic V, Verani CN and Dou QP. Inhibition of the proteasome activity by organic gallium complexes contributes to their anti-prostate tumor activities. 7th Annual Symposium, Michigan Prostate Research Colloquium, Basic and Clinical Advances in Prostate Cancer Research, Henry Ford Health System, One Ford Place, Detroit, MI, May 12, 2007.
- Vesna Milacic, Di Chen, Luca Ronconi, Kristin R. Landis-Piwowar, Dolores Fregona, and Q. Ping Dou. Potential use of gold(III) dithiocarbamates as proteasome inhibitors and apoptosis inducers for breast cancer treatment. Wayne State University Graduate Student Research Day, Detroit, MI, September 20, 2007 (the first place in the oral session).
- Frezza M, Chen D, Shakya R, Cui QZC, Milacic V, Verani CN and Dou QP. Gallium(III) complexes as proteasome inhibitors and apoptosis inducers in prostate cancer cells. Wayne State University Graduate Student Research Day, Detroit, MI, September 20, 2007.
- Sarmad Sahiel Hindo, Michael Frezza, Dajena Tomco, Rama Shanmugam, Q. Ping Dou, Claudio N. Verani. Syntheses, Structure, and Proposed Biological Mechanism for Anticancer Activity of various Ni(II), Cu(II), Zn(II) Complexes. Wayne State University Ninth Graduate Research Symposium 2007, Detroit, MI, October 6, 2007.

INVITED ORAL PRESENTATIONS (IN THE LAST FIVE YEARS):

- Dou QP. Apoptosis control and cancer. Department of Pharmacology, University of Pittsburgh School of Medicine, February 7, 1997
- Dou QP. Apoptosis control and cancer. *Cephalon, Inc.*, March 11, 1997
- Dou QP. RB and apoptosis. University of Pittsburgh Cancer Institute, Molecular Oncology Seminar Series, April 16, 1997
- Dou QP. Activation of apoptotic death program in human cancer. H. Lee Moffitt Cancer Center & Research Institute at the University of South Florida, April 28, 1997
- Dou QP. Invited Speaker. Retinoblastoma protein and the regulation of apoptosis. 7th SCBA International Symposium, Toronto, Canada, July 6-11, 1997.**
- Dou QP. Cell cycle and Apoptosis. University of Pittsburgh Cancer Institute, FAS-L Club, August 12, 1997.
- Dou QP. Apoptosis regulation in breast cancer. Second Annual Pittsburgh Minisymposium on Basic and Translational Research in Breast Cancer, Center for Environmental and Occupational Health and Toxicology, University of Pittsburgh, August 16, 1997
- Dou QP. Invited speaker. RB and apoptosis control. Center for Clinical Pharmacology, University of Pittsburgh School of Medicine, September 25, 1997

Dou QP. Invited Speaker. Targeting the Apoptotic Signaling Pathway in Human Cancer. Departments of Biochemistry & Molecular Biology and Microbiology & Immunology, University of North Texas Health Science Center at Fort Worth, September 29, 1997

Dou QP. Invited Speaker and Session Chairman. Putative roles of retinoblastoma protein in apoptosis. 2nd World Congress on Advances in Oncology, Athens, Greece, 16-18 October, 1997

Dou QP. Invited Speaker. Cell cycle checkpoint proteins as apoptosis therapeutic targets. NATO Advanced Research Workshop at H. Lee Moffitt Cancer Center and Research Institute, University of South Florida, Protein-Protein and Protein-Lipid Interactions in Signal Transduction: Use of Small Synthetic Molecules as probes and Therapeutic Agents, Clearwater Beach, Florida, December 5-9, 1997

Dou QP. Targeting ubiquitin/proteasom-mediated protein degradation pathway in human cancers. Research Progress Seminar Series at H. Lee Moffitt Cancer Center and Research Institute and University of South Florida, Tampa, Florida, October 29, 1998

Dou QP. Invited Speaker. Targeting ubiquitin/proteasom-mediated protein degradation pathway in human cancers. Department of Pharmacology and Therapeutics, University of South Florida College of Medicine, Tampa, Florida, February 17, 1999

Dou QP. Invited Speaker. Bax degradation by the proteasome: a survival mechanism used by human cancer cells. Department of Biochemistry & Molecular Biology, University of South Florida College of Medicine, Tampa, Florida, October 15, 1999

Gao G and Dou QP. G1 phase-dependent Bcl-2 expression correlates with chemoresistance of human cancer cells. Oral presentation. 29th Annual Scientific Meeting of the International Society for Experimental Hematology, Tampa, FL, July 8-11, 2000

Smith DM and Dou QP. Green tea targets human tumor cell DNA synthesis and consequently induces apoptosis. Poster presentation. New Molecular Targets for Cancer Therapy, St. Petersburg Beach, FL, October 14-17, 2000

Dou QP. Invited Speaker. Proteasome inhibitors as novel anticancer drugs. Cancer Research and Biotechnology in the I-4 Corridor, Moffitt Cancer Center & Research Institute, Tampa, Florida, August 21, 2000

Dou QP. Invited Speaker. Therapeutic potential of proteasome inhibitors in cancer prevention and treatment. Moffitt Cancer Center Research Retreat, Saddlebrook Resort, FL, May 19, 2001

Smith DM and Dou QP. Drug Discovery: Hunting for Cancer-Specific Molecular Targets - from Natural to Synthetic Compounds. Drug Discovery and Developmental Therapeutics Seminar, Moffitt Cancer Center & Research Institute, June 21, 2001

Dou QP. Invited Speaker. Proteasome inhibitors. New drugs in hematologic malignancies, Institute Of Hematology and Medical Oncology, "Seragnoli", University of Bologna, Bologna, Italy, November 12-14, 2001

Dou QP. Invited Speaker. Proteasome: a novel target for cancer prevention and treatment as well as anti-angiogenic therapy. Moffitt Grand Rounds, Moffitt Cancer Center & Research Institute, Tampa, FL, November 15, 2002

Dou QP. Invited Speaker. Identification of A Novel Molecular target for Anti-Copper and Anti-Angiogenic Therapies. Attenuon, L.L.C., San Diego, CA, November 25, 2002

Dou QP. Invited Speaker. Natural Proteasome Inhibitors and Chemoprevention. Karmanos Cancer Institute at Wayne State University, Detroit, MI, February 6, 2003

Dou QP. Invited Speaker. Proteasome, a novel target for anti-cancer drug discovery. Moffitt Scientific Retreat, Tampa, FL, February 15, 2003

Dou QP. Invited Speaker. Proteasome Inhibitors. Sopherion Therapeutics, Inc., New Haven, CT, March 13, 2003

Dou QP. Invited Speaker. TBN. Department of Pathology, Wayne State University, Detroit, MI, June 25, 2003 (rescheduled)

- Daniel KG and Dou QP. Organic-copper complexes as a new class of proteasome inhibitors: the potential of converting a pro-angiogenic factor to a cancer cell-specific killer. Drug Discovery and Developmental Therapeutics Seminar, Moffitt Cancer Center & Research Institute, May 29, 2003
- Dou QP. Invited Speaker. Chemoprevention: targeting the proteasome. Karmanos Scientific Retreat, Detroit, MI, August 22, 2003
- Dou QP. Invited Speaker. The proteasome: a novel molecular target for cancer prevention and treatment. The Protease Group, Karmanos Cancer Institute, Detroit, MI, September 2, 2003
- Dou QP. Invited Speaker. Proteasome inhibitors and chemoprevention. Great lakes chemoprevention retreat, Maumee Bay Resort, Ohio, September 13, 2003
- Dou QP. Invited Speaker. Prostate Cancer Research Summary. Henry Ford Health System/WSU Prostate Journal Club, Detroit, MI, November 12, 2003
- Dou QP. Invited Speaker. Green tea and cancer prevention. Presentation to Cancer Biology Candidate Students, Karmanos Cancer Institute, Detroit, MI, April 3, 2004
- Dou QP. Invited Speaker. Synthetic beta-lactam antibiotics and a selective breast cancer cell apoptosis inducer: Significance in breast cancer prevention and treatment. The Breast Cancer Group, Karmanos Cancer Institute, Detroit, MI, May 6, 2004
- Dou QP. Invited Speaker. Tea Polyphenols. Karmanos Cancer Institute Research Retreat, Detroit, MI, August 27, 2004
- Dou QP. Invited Speaker. Discovery of Novel Small Molecules: Rational Design, Structure-Activity Relationships, Cellular Targets, and Potential Uses for Cancer Treatment and Prevention. The Developmental Therapeutics Group, Karmanos Cancer Institute, Detroit, MI, September 8, 2004
- Dou QP. Invited Speaker. Proteasome Inhibitors: Killing *via* Tumor-Specific Signaling. Basic and Translational Aspects of Cancer Cell Signaling Research Retreat, Karmanos Cancer Institute, Detroit, MI, January 14, 2005
- Dou QP. Invited Speaker. Searching for Natural and Pharmacological Proteasome Inhibitors for Cancer Prevention and Treatment. Department of Pharmaceutical Sciences, Eugene Applebaum College of Pharmacy and Health Sciences, Wayne State University, Detroit, MI, April 13, 2005
- Dou QP. Invited Speaker. Searching for Novel Polyphenol Proteasome Inhibitors for Cancer Prevention and Treatment. Department of Urology at the University of California San Francisco and San Francisco VA Medical Center, San Francisco, CA, April 28, 2005
- Dou QP. Invited Speaker. Roles of polymorphic catechol-O-methyltransferase gene, tea polyphenols and proteasome in cancer prevention. Population Studies and Prevention Joint Meeting, Karmanos Cancer Institute, Detroit, MI, June 14, 2005
- Dou QP. Invited Speaker. Tea polyphenols, Proteasome and Polymorphic Catechol-O-Methyltransferase: Use in Cancer Molecular Diagnosis, Prevention and Treatment. Department of Chemistry at McGill University and American Diagnostica Inc., Montreal, Quebec, Canada, August 22, 2005
- Kristin Landis (Mentor: Dou QP). Selected Speaker. Molecular Studies for the Regulation of Green Tea Polyphenol Biological Function by the Polymorphic Gene Product Catechol-O-Methyltransferase. Wayne State University Graduate Student Research Day, Detroit, MI, September 22, 2005
- Dou QP. Invited Speaker. Copper as a novel target for determining fate of AR and prostate cancer cells. Karmanos Cancer Institute Research Retreat, Detroit, MI, October 7, 2005
- Dou QP. Searching for natural proteasome inhibitors for cancer prevention and anti-cancer drug discovery. Department of Pathology Retreat, Wayne State University School of Medicine, Detroit, MI, August 27, 2005 (canceled)
- Landis-Piwowar KR, Wan SB, Daniel KG, Chen D, Chan TH, and Q. Ping Dou. Computational Modeling and Biological Evaluation of Methylated (-)-EGCG Analogs. Selected as a

- Minisymposium presentation. The 2006 AACR 97th Annual Meeting, Washington DC, April 1-5, 2006
- Dou QP. Invited Speaker. Roles of Diet, Biometals, and Environmental Factors in Cancer Prevention. Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, China, April 19-20, 2006
- Dou QP. Invited Speaker. Nutrient-Gene-Environment Interactions and Molecular Prevention of Cancer. The Institution of Environmental Sciences, Shandong University, Jinan, Shandong, China, April 24, 2006
- Dou QP. Invited Speaker. Discovery of Novel Small Molecules for Cancer Therapies: - *From Nature to Laboratories and ... back*. The Institution of Environmental Sciences, Shandong University, Jinan, Shandong, China, April 25, 2006
- Dou QP. Invited Speaker. A Common Target of Dietary Factors, Traditional Medicine and Chemopreventive Agents in Human Prostate Cancer: the Significance in Molecular Prevention. 6th Annual Symposium, Michigan Prostate Research Colloquium, Basic and Clinical Advances in Prostate Cancer Research, University of Michigan Medical School, Towsley Center, Dow Auditorium, Ann Arbor, MI 48109, May 6, 2006
- Dou QP. Invited Speaker. Molecular Prevention of Human Cancer: An Example of Diet-Gene-Environment Interaction. Institution of Environmental Health Sciences, Wayne State University, Detroit, MI, June 15, 2006.
- Dou QP. Invited Speaker. A Lesson Learned from Thymidine Kinase Transcription at G₁/S and later Stories. Symposium Honoring Dr. Pardee on the occasion of his 85th birthday. Boston, MA, June 24, 2006.
- Dou QP. Invited Speaker. Discovery of Novel Natural and Synthetic Compounds for Molecular Prevention of Human Cancer. Henry Ford Health Systems, Detroit, MI, August 3, 2006.
- Dou QP. Invited Speaker. Green Tea and Cancer Prevention. The Day of Wellness, Grosse Pointe War Memorial, Grosse Pointe, MI, September 16, 2006.
- Kristin Landis (Mentor: Dou QP). A Novel Pro-drug of Green Tea Catechin (-)-EGCG as a Potential Anti-Breast Cancer Agent. Wayne State University Graduate Student Research Day, Detroit, MI, September 21, 2006. (Session Winner)
- Dou QP. Invited Speaker. Green Tea and its Role in Cancer Prevention. Dow and Towsley Foundation Visit, Karmanos Cancer Institute Research, Detroit, MI, October 11, 2006
- Dou QP. Invited Speaker. Molecular Cancer Prevention and Therapies. Shandong Institute of Cancer Prevention and Treatment, Jinan, Shandong, China, October 18, 2006
- Dou QP. Invited Speaker. Discovery of Novel Small Molecules for Cancer Prevention and Therapies. Wuhan Botanical Garden/ Wuhan Institute of Botany, The Chinese Academy of Sciences, Wuhan, China, October 20, 2006
- Dou QP. Invited Speaker. The Proteasome as a Potential Cellular Target of Organic Toxic Metals. The 3rd International Symposium on Persistent Toxic Substances, Beijing, China, October 22-25, 2006**
- Dou QP. Invited Speaker. Proteasome inhibitors for cancer prevention and treatment. Karmanos Cancer Institute the Protease and Cancer Program Annual Retreat, Scott Hall, Wayne State University, Detroit, MI, December 1, 2006
- Dou QP. Invited Speaker. Converting the proangiogenic copper to a specific death inducer: significance in breast cancer prevention and treatment. Karmanos Cancer Institute the Breast Cancer Program Annual Retreat, HWRC 2nd Floor auditorium, Karmanos Cancer Institute, Wayne State University, Detroit, MI, March 2nd, 2007
- Dou QP. Invited Speaker. Molecular Prevention of Human Cancer: A Lesson Learned from Diet-Gene-Environment Interaction. Environmental Toxicology Graduate Program and Department of Chemistry, University of California at Riverside, Riverside, CA, April 18, 2007.**

Dou QP. Invited Speaker. Molecular Prevention of Human Cancer: Role of Diet-Gene-Environment Interactions. College of Chemistry, Central China Normal University, Wuhan, China, June 29, 2007

Dou QP. Invited Speaker. Proteasome Inhibition as a Novel Strategy for Cancer Therapies. The Fourth Wuxi People's Hospital affiliated to Suzhou University, Wuxi, Jiangsu 214062, P. R. China, July 2, 2007

Dou QP. Invited Speaker. Molecular Prevention of Human Cancer: Role of Diet-Gene-Environment Interactions. School of Medicine, Shandong University, Jinan, Shandong, China, July 4, 2007

Dou QP. Invited Speaker. Molecular Prevention of Human Cancer: Role of Diet-Gene-Environment Interactions. The College of Chemistry and Chemical Engineering, Ocean University of China, Qingdao, China, July 9, 2007

Dou QP. Invited Speaker. Molecular Prevention of Human Cancer: Role of Diet-Gene-Environment Interactions. The College of Medicine, Ocean University of China, Qingdao, China, July 10, 2007

Dou QP. Invited Speaker. Targeting the Tumor Cell Proteasome with Natural Inhibitors for Cancer Prevention and Treatment. School of Pharmaceutical Sciences, Shandong University, Jinan, Shandong, China, July 11, 2007

Dou QP. Invited Speaker. Molecular Prevention of Human Cancer: Role of Diet-Gene-Environment Interactions. Shandong Academy of Medical Sciences, Jinan, Shandong, China, July 13, 2007

Dou QP. Invited Speaker. Molecular Prevention of Human Cancer: Role of Diet-Gene-Environment Interactions. Department of Pathophysiology, Guangzhou Medical College, Guangzhou, China, July 19, 2007

Dou QP. Invited Speaker. New use of old copper-binding drugs: converting the proangiogenic copper to a specific cancer cell death inducer. Molecular Therapeutics of Cancer, Gordon Research Conference, Colby-Sawyer College, New London, New Hampshire, July 22-27, 2007.

Vesna Milacic (Mentor: Dou QP). Potential use of gold(III) dithiocarbamates as proteasome inhibitors and apoptosis inducers for breast cancer treatment. Wayne State University Graduate Student Research Day, Detroit, MI, September 20, 2007.

Frezza M, (Mentor: Dou QP). Gallium(III) complexes as proteasome inhibitors and apoptosis inducers in prostate cancer cells. Wayne State University Graduate Student Research Day, Detroit, MI, September 20, 2007.

Dou QP. Invited Speaker. Converting the proangiogenic copper to a specific cancer cell death inducer: importance of tumor tissue copper mapping. The joint XOR/BioCAT Microprobe Workshop, Northwestern University, Chicago, IL, November 15-16, 2007

Dou QP. Invited Speaker. TBA. Karmanos Cancer Institute, Prostate Cancer Working Group (PCWG), HWRC 4th Floor Boardroom, Karmanos Cancer Institute, Wayne State University, Detroit, MI, March 21st, 2008

Dou QP. Invited Speaker. Green Tea Polyphenols as a Natural Tumor Cell Proteasome Inhibitor. The 3rd International Symposium on Functional Foods, PLANT POLYPHENOS, "What would life be without plant polyphenols?" Hong Kong Polytechnic University, Hung Hom, Kowloon, Hong Kong SAR, P.R. China, April 03-05 2008

Dou QP. Invited Speaker. Targeting the Tumor Cell Proteasome with Small Molecule Inhibitors. Department of Chemical Biology and Therapeutics, St. Jude Children's Research Hospital, Memphis, TN, May 5, 2008

Dou QP. Invited Speaker. Molecular Mechanisms of Green Tea Polyphenols. The International Conference on Nutrition and Cancer, Antalya, Turkey, May 20-23, 2008